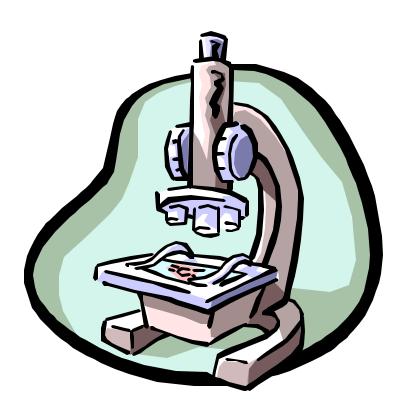
# Research in Progress

2 0 0 1



Research and Development Service (584/151)

**Department of Veterans Affairs** 

VA Medical Center 601 Highway 6 West Iowa City, Iowa

http://www.icva.gov Ph: 319-339-7151

## TABLE OF CONTENTS:

Investigator	Title	Page
Lee-Ann H. Allen, Ph.D.	Interaction of Helicobacter Pylori with Human Neutrophils and Macrophages	1
Robert F. Ashman, M.D.	Regulation of Multiple Stages of Lymphocyte Apoptosis: Effects of Bacterial DNA	1
Zuhair K. Ballas, M.D.	Oligodeoxynucleotides as Cancer Immunotherapeutic Agents	2
Robert S. Bar, M.D.	Vascular Disease in Diabetes: Metabolic Basis	3
Robert S. Bar, M.D.	Vascular Endothelium and the IGFs	3
Gail A. Bishop, Ph.D.	Regulation of B Lymphocyte Function and B cell Transformation	4
Bradley E. Britigan, M.D.	Promotion of Hydroxyl Radical-mediated Tissue Injury by Pseudomonas Secretory Products	4
Bradley E. Britigan, M.D.	Biology of Macrophage Transferrin-independent Fe Update	5
Joseph Buckwalter, M.D.	Alterations in Cartilage Extracellular Matrix and the Etiology of OA	6
John J. Callaghan, M.D.	Biomechanics of Total Hip Dislocation	6
Mark W. Chapleau, Ph.D.	Baroreflex and Autonomic Dysfunction in Atherosclerosis and Hypertension	7
Michael B. Cohen, M.D.	Cell Death in Human Prostate Cancer	8
Brian L. Cook, D.O.	A Prospective Outcome Study In Alcoholism	8
Robert T. Cook, M.D.,	Cellular Immune Function and Phenotype in Multiple	9

Research in Progress 2001, VAMC Iowa City, IA: page i

Ph.D.	Myeloma	
Robert T. Cook, M.D., Ph.D.	Alcoholism: Modulation and Function of Lymphocyte Subsets (Clinical Immunology of Alcoholism)	9
Robert T. Cook, M.D., Ph.D.	Alcohol Effect on HIV Infectivity and Cellular Responses	10
John S. Cowdery, M.D.	Bacterial DNA Induction in Macrophage/Dendritic Cell IL-12 Production	11
Joseph J. Cullen, M.D.	Control of Esophageal Motor Function: Roles of Antioxidant Enzymes and Superoxide	11
Joseph J. Cullen, M.D.	The Role of Free Radicals in Biliary Tract Motility	12
Kevin Dellsperger, M.D.	Mechanisms of Coronary Microvascular Regulation	13
Kevin Dellsperger, M.D.	Mechanisms of Diabetic Endothelium Dysfunction	13
G.M. Denning, Ph.D.	Cytokine-dependent Regulation of the Polymeric Iga Receptor in Human Intestinal Epithelial Cells	14
G.M. Denning, Ph.D.	Mechanisms By Which Pseudomonas Aeruginosa Secretory Factors Contribute to Airway Disease	14
Gerald F. DiBona, M.D.	Differentiated Sympathetic Neural Control of the Kidney	14
Joseph S. Dillon, M.D.	Development of Novel Genes for Type II Diabetes Using the GK Rat Model	15
Bradley S. Dixon, M.D.	Growth Factor Regulation of the Bradykinin Receptor in Vascular Injury	15
Bradley N. Doebbeling, M.D., MSc	Epidemiology and Control of Antimicrobial Resistance in Hospitals	16
Bradley N. Doebbeling, M.D., MSc	Determinants of Clinical Guideline Implementation Effectiveness	17
K.S. Ephgrave, M.D.	Acute Gastric Mucosal Injury and the Gastric Responses to Stress	18

Research in Progress 2001, VAMC Iowa City, IA: page ii

Bernard Fallon, M.D.	Prostate Intervention vs. Observation Trial (PIVOT)	18
Robert Felder, M.D.	Petidergic Mechanisms in the Nucleus of the Solitary Tracts	19
Elizabeth H. Field, M.D.	CD4 Regulatory Cells in Immune Tolerance	19
F. Jeffrey Field, M.D.	Regulation of Intestinal Cholesterol Metabolism	20
Sue E. Gardner, Ph.D., RN	The Validity of the Clinical Signs and Symptoms Used to Identify Localized Chronic Wound Infection	20
Michael Garvey. M.D.	Naturalistic Versus Intensive Continuation Treatment of Mood Disorders	21
Lois J. Geist, M.D.	Macrophage Function in the Pathogenesis of CMV Infection	21
Sarah L. George, M.D.	Virologic and Immunologic Analysis of Sero-Silent Hepatitis C Infection	22
Nancy E. Goeken, Ph.D.	The Immunobiology of Bone Marrow Transplantation	23
Thomas H. Haugen, M.D., Ph.D.	Regulation of Bovine Papillomavirus -1 Early Gene Expression	23
William G. Haynes, M.B.Ch.B., M.D.	Hyperglycemia, Insulin Resistance and Endothelial Function in Human Diabetes	24
Donald D. Heistad, M.D.	SOD Gene Transfer and Vascular Function	25
Joseph A. Hill, M.D., Ph.D.	Voltage-Dependent K <sup>+</sup> Channels in the Cardiovascular System: Kv1.5 a -Subunit	25
Daniel G. Hug, B.S., Ph.D.	Role of Urocanic Acid in Immunosuppression by Ultraviolet Radiation	26
Gary W. Hunninghake, M.D.	Silica or Asbestos Upregulation Tumor Necrosis Factor Gene	26
Daniel Kahn, M.D.	Phase I/II Study of Sm-153-Lexidronam for the Treatment of Patients with Refractory Rheumatoid Arthritis	27

Randy H. Kardon, M.D.	Objective Testing of the Visual Field Using Pupillary Contraction to Light: Comparison of Pupil and Visual Responses	27
Lawrence P. Karniski, M.D.	Identification and Characterization of a Human Renal Sulfate Transporter	28
Louis V. Kirchhoff, M.D., M.P.H.	Recombinant Assays for Diagnosing Chagas Disease	28
Ulla C. Kopp, Ph.D.	Afferent Renal Nerves: Role of Neurotransmitters	29
Arthur M. Krieg, M.D.	B Cell Activation by Oligodeoxynucleotides with Cpg Motifs	30
David J. Kusner, M.D.	The Regulation of Phospholipase D in Phagocytic Leukocytes	30
Kathryn Lamping, Ph.D.	Responses of the Coronary Circulation in Enos-deficient Mice	31
Hon-Chi Lee, M.D., Ph.D.	Mechanism of Alpha-2 Adrenergic Modulation of Cardiac Electrophysiology	32
Steven R. Lentz, M.D., Ph.D.	Vascular Dysfunction in Hyperhomocysteinemia and Atherosclerosis	32
Ramon Lim, M.D., Ph.D.	GMF as a Signaling Molecular	33
David M. Lubaroff, Ph.D.	Inhibition of CD40-CD40L interactions augments vaccine therapy of prostate cancer	33
David M. Lubaroff, Ph.D.	VA Research Enhancement Award Program (REAP): Cellular Activation in Prostate Cancer	34
Donald Macfarlane, M.D.	Genetic Expression Changes in B-Cells with Exposure to Bacterial DNA	35
Donald Macfarlane, M.D.	Inhibition of Bacterial DNA Induced Immune Response	35
Rama Mallampalli, M.D.	Lipid Regulation of Cytidylyltransferase in Adult Lung	36
James B. Martins, M.D.	Purkinje Origin of Ventricular Tachycardia	36

Research in Progress 2001, VAMC Iowa City, IA: page iv

Nina A. Mayr, M.D.	Radiation Therapy Oncology Group (RTOG)	37
Michael McCormick, Ph.D.	Eosinophil-mediated Formation of Reactive Tyrosine Species	37

Research in Progress 2001, VAMC Iowa City, IA: page v

Stephen E. McGowan, M.D.	Influences of the Extracellular Matrix on Elastin Production	38
William M. Nauseef, M.D.	Neutrophil Myeloperoxidase: Structure, Function, and Biosynthesis	39
William M. Nauseef, M.D.	p57, a Coronin Homologue and Actin-Binding Protein Important for Assembly of the NADPH Oxidase of Human Neutrophils	39
Gary E. Rosenthal, M.D.	Impact of Outsourcing VA Cardiac Surgery on the Cost and Quality of Care	40
James Rossen, M.D. et al.	Clinical Outcomes Using Revascularization and Aggressive Drug Evaluation (The COURAGE Trial)	41
Anne Sadler, R.N., Ph.D.	Sexual Victimization and the Military Environment: Contributing Factors, Vocational, Psychological and Medical Sequelae	41
Larry S. Schlesinger, M.D.	Biology of Tuberculosis and Macrophage Membrane Receptors	42
Warren Schmidt, M.D., Ph.D.; Douglas La Brecque, M.D.	A Multi-center Trial to Evaluate the Epidemiology, Natural History, and Treatment Response of Hepatitis C in the United States Veterans Population	42
Warren Schmidt, M.D., Ph.D.; Jack Stapleton, M.D.; Douglas La Brecque, M.D.	Evaluation of Hepatitis Viruses in Peripheral Blood and Liver Biopsy Tissue of Individuals with Chronic Hepatitis	43
Konrad Schulze, M.D., FRCP	Mechanics of Stomach and Small Intestine	43
William L. Sivitz, M.D.	Hormonal and Metabolic Regulation of Glucose Transport and GLUT-1 Expression in Endocrine Responsive Breast cancer cells	44
William L. Sivitz, M.D.	Metabolic Interaction of Insulin and Leptin and the Regulation of Nerve Activity and Vascular Reactivity	44

Jack Stapleton, M.D.	Hepatitis C Projects	45
Jack Stapleton, M.D.	HIV Studies	46
John B. Stokes III, M.D.	Mechanisms of Steroid Hormone Effects on Ion Transport	46
William T. Talman, M.D.	Cardiovascular Responses to Glutamate Linked to Nitric	47
William T. Talman, M.D.	Oxide  Pathways Connecting Cardiovascular Regions of the Nucleus Tractus Solitarii with the Superior Salivatory Nucleus	47
Lubomir P. Turek, M.D.	Human Papillomavirus -16 Gene Regulation in Cervical Cancer	48
Bonnie Wakefield, Ph.D., R.N. and James Flanagan, M.D., Ph.D.	Implementation and Evaluation of Telemedicine to Improve Health Care	48
Bonnie Wakefield, Ph.D., R.N. et al.	Predicting Impending Dehydration in Elderly Veterans	49
Bonnie Wakefield, Ph.D., R.N.	Preventing Functional Decline in Hospitalized Elderly	50
Michael Wall, M.D.	Motion Perimetry: A New Method for Early Detection of Visual Loss	51
Jerrold Weiss, Ph.D.	Neutrophils and Bacterial Phospholipid Degradation	51
Jerrold Weiss, Ph.D.	Microbicidal Activity of Leukocytes: Active Factors	52
Jerrold Weiss, Ph.D.	Mobilization and Delivery of Meningococcal Lipoligosaccharides to Host Targets	53
Mary E. Wilson, M.D.	Interactions of Leishmania Chagasi with Host Macrophages	53
Patricia L. Winokur, M.D.	Analysis of Human Papillomavirus E2 Protein Interaction	54
Catherine Woodman, M.D.	The Relationship Between Fibromyalgia and Psychiatric Disorders in Persian Gulf War Veterans: A Family Study	54
Mark A. Yorek, Ph.D.	Circulating Factors in the Etiology of Diabetic Vascular	55

Research in Progress 2001, VAMC Iowa City, IA: page vii

Disease

PRINCIPAL INVESTIGATOR:

Lee-Ann H. Allen, Ph.D.

### PROJECT TITLE:

Interaction of Helicobacter Pylori with Human Neutrophils and Macrophages

### **SUMMARY:**

About half of the world's population is infected with the bacterium Helicobacter pylori. These bacteria live in the stomach and cause inflammation of the stomach lining (gastritis) which is usually asymptomatic. Infection with *H*. pylori is the major cause of stomach ulcers and intestinal (duodenal) ulcers. In addition, infection with *H. pylori* is a risk factor for the development of stomach cancers such as gastric adenocarcinoma and gastric lymphoma. Once H. *pylori* is established in the stomach the bacteria persist for life and are not removed by the response of the human immune system. Nevertheless, only about 10% of all people infected with *H. pylori* will develop ulcers or stomach cancer. It is believed that both bacterial and host (human) factors contribute to the development of disease, however, the steps leading to disease are only beginning to be explored. During an immune response macrophages and neutrophils are recruited from the blood stream to the site of infection; in the case of *H. pylori* this site is the stomach. Both macrophages and neutrophils are phagocytes (which means "big eaters"), and they normally help remove invading bacteria by ingesting (phagocytosing or "eating") them. Phagocytosis occurs after a bacterium binds to the surface of a macrophage or neutrophil. The membrane of the phagocyte then wraps around the bacterium in response to specific localized signals. Once inside the macrophage or neutrophil, bacteria are normally killed by a combination of reactive oxygen species (hydrogen peroxide, superoxide, and hydroxyl radicals), and digestive enzymes that are stored in an intracellular compartment called a lysosome. My laboratory has discovered that, in contrast to most bacteria, H. pylori are able to avoid being killed by macrophages. H. pylori bind avidly to the macrophage surface and are ingested, however, once inside the phagocyte

these bacteria are not killed, rather they produce a toxin which allows bacteria to survive and eventually kill the macrophage. The toxin, VacA, acts on an unidentified target inside the macrophage and alters the composition of intracellular membranes called endosomes and lysosomes. As a result, the ingested H. pylori avoid contact with lysosomes and are not killed. After several hours the toxin kills the macrophage and live *H. pylori* are released. An understanding of H. pylori-phagocyte interactions at the molecular level is a necessary first step toward the development of novel therapies for Helicobacter infection and may allow us to define which infected persons are more likely to develop ulcers or cancer. Ulcers are a significant health problem for both veterans and the general population and it is possible that the knowledge gained from this research will result in better treatment for *H. pylori* infection and consequently reduce the number of persons with ulcers or stomach cancer.

MeSH terms: Helicobacter pylori; ulcers; inflammation; macrophages; neutrophils; phagocytosis.



### PRINCIPAL INVESTIGATOR:

Robert F. Ashman, M.D.

### PROJECT TITLE:

Regulation of Multiple Stages of Lymphocyte Apoptosis: Effects of Bacterial DNA

### **SUMMARY:**

The number of cells of each type that make up our bodies is determined by the rate of cell proliferation (growth) and the rate of cell death (apoptosis). Cells that lose the ability to die, especially if they also retain the ability to grow, may form a cancer. In the development of the immune system, the lymphocytes that can potentially make immune responses to selfantigens are normally signaled to die, and if they

live instead, autoimmunity may result. Cancer and autoimmune diseases like rheumatoid arthritis and lupus afflict many VA patients. We are investigating how the cells of the immune system choose between life and death as they respond to their environment.

The body considers the DNA (the genetic material) of bacteria as a danger signal, which jump-starts the immune system, amplifying certain normal immune responses. We have identified the precise structures of the DNA pieces required for this activity. B cells are the parents of antibody-making cells. We have shown that short bits of bacterial DNA block B cell death programs causing tremendous growth and activity. More recently we have identified DNA structures, closely related to the sequences that turn on B cells, which do the opposite, preventing B cells from being turned on by bacterial DNA. Identifying how inhibitory and stimulatory DNA sequences interact with molecules in the B cell that control the death and growth programs will be important for exploiting the immune stimulatory properties of bits of bacterial DNA in designing new vaccines, and in preventing cancer or asthma.

MeSH terms: immunology; rheumatology; lymphocyte; apoptosis; cell death; DNA.



PRINCIPAL INVESTIGATOR: Zuhair K. Ballas, M.D.

PROJECT TITLE:

Oligodeoxynucleotides as Cancer Immunotherapeutic Agents

### **SUMMARY:**

Natural killer (NK) cells are large granular lymphocytes that occur spontaneously and do not require prior sensitization for the expression of their function. NK cells have many functions

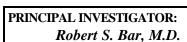
that include their ability to kill certain tumors and their ability to secrete certain substances, called cytokines or lymphokines, which are proteins known to regulate various aspects of the immune system. NK cells, as well as some T lymphocytes, can expand their anti-tumor killing activity upon stimulation with lymphokines. Lymphokine-activated killer (LAK) cells can kill NK-sensitive and NK-resistant tumors as well as autologous lymphocytes that have been virally infected or chemically modified. The LAK phenomenon has been at the basis of the development of tumor immunotherapy protocols that utilize various biological response modifiers (BRM) with interleukin-2 (IL-2) being at the forefront.

It has been known for some time that mycobacteria (the family of bacteria that cause tuberculosis) are potent boosters of the immune system. More recent evidence suggests that mycobacterial DNA as well as bacterial DNA may serve as potent stimulators of some immune functions. Mammalian DNA, on the other hand, does not display such effects. The major difference between bacterial and mammalian DNA is the preponderance of unmethylated CpG (C stands for cytosine, G stands for guanosine; these are two of the four building blocks of DNA) motifs in the former. We have recently demonstrated that oligodeoxynucleotides (ODN) with the CpG motif are potent inducers of NK, in vivo and in vitro, as well as LAK activity.

The hypothesis of this proposal is that ODN with the CpG motif are potent biological response modifiers, which by virtue of their induction of NK and LAK activity, are effective in treating cancer. The early focus of this proposal is examination of the optimal parameters for, and the mechanisms of, the induction of NK and LAK activity *in vivo* and *in vitro*. The possible synergy of CpG ODN with other cytokines in the induction of LAK activity will also be examined. The ability of CpG ODN to inhibit the growth of newly introduced tumors and their ability to induce regression of established tumors will be examined in a murine model.

Our experiments are expected to define the potential role of these novel biological response modifiers in tumor immunotherapy either on their own or in conjunction with other immune modulators.

MeSH terms: cancer; immunotherapy; immunology; cytokines; NK cells; LAK cells.



PROJECT TITLE:

Vascular Disease in Diabetes: Metabolic Basis

### **SUMMARY:**

Diabetes mellitus is a disease characterized by elevated levels of blood sugar and abnormalities of large and small blood vessels. The vascular abnormalities lead to complications affecting vision, kidney function, the nervous system and an increased frequency of heart attacks, stroke and peripheral vascular disease. One of the first vascular abnormalities noted in both Type I and Type II diabetes is an impairment in the ability of blood vessels to dilate appropriately, which is thought to lead to the more serious vascular diseases. This Center proposal uses a multidisciplinary approach aimed at defining the mechanisms underlying the early vascular defects in diabetes and designing therapies that will prevent their occurrence or lessen their impact on the more severe, subsequent vascular disease. Primary emphasis is given to studies of humans with diabetes. The human studies have all been coordinated through the patient core, which includes three certified diabetes nurse educators who have recruited all patients for the studies and worked with the CRC personnel and principal investigators to perform the required studies. Animal models of diabetes as well as cultured vascular cells will also be studied.

Mesh terms: diabetes; vascular dysfunction.

PRINCIPAL INVESTIGATOR: Robert S. Bar, M.D.

PROJECT TITLE:

Vascular Endothelium and the IGFs

### **SUMMARY:**

Diabetes is associated with abnormalities of the insulin-like growth factors (IGFs) and their binding proteins. The IGFs can mediate several of the metabolic and growth promoting effects of insulin. The clinical effects of the IGFs are determined by their associations with six specific IGF binding proteins which can both inhibit and potentiate the effects of the IGFs. Our laboratory has focused on the interactions of the vascular endothelium and the insulin-like growth factors (IGFs). We have shown that: 1) type 1 and type 2 IGF receptors are present in cultured endothelial cells (EC) and in capillary endothelium of perfused organs of the intact rat; 2) the IGFs potently stimulate acute metabolic processes in cultured microvessel EC and stimulate the synthesis of sulfated proteoglycans in all cultured EC; 3) cultured EC process IGF-I and IGF-II by distinct processes which result in the cell storing and releasing intact IGF-I while progressively degrading IGF-II; 4) all cultured EC have specific mRNA for 5 of the 6 known IGF binding proteins (IGFBPs) with microvessel cells predominantly synthesizing and releasing IGFBP-2 and -3 while EC cultured from large blood vessels make IGFBP-4; 5) IGFBP-3 and -5 specifically bind to the endothelial cell surface and extracellular matrix (ECM) with binding dependent on a heparin binding region near the carboxy terminus of the protein; 6) synthetic 18 amino acid peptides that include the heparin-binding domain compete for IGFBP-3 and -5 binding to the cells, directly bind to the ECM and stimulate acute metabolic processes in

microvessel EC, a bioactivity that is not shared by the intact, parent IGFBP-3 or - 5; 7) IGFBP-3 is degraded by proteases that are present at the endothelial surface generating fragments of IGFBP-3, some of which have bioactive properties not present in the parent IGFBP-3 molecule; 8) growth factors implicated in the pathogenesis of several diabetic complications can directly stimulate (IGF-I) or inhibit (TGF-,8) the endothelial gene for IGFBP-3, the major circulating IGFBP; and 9) the bovine gene for IGFBP-3 has been cloned and regions of the gene encoding the IGF-I and TGF-,8 responses have been tentatively identified. These findings have been used to develop a working model that accounts for many of the known autocrine, paracrine and endocrine functions of the IGFs which are known to be altered in human diabetes and animal models of the disease. It is hoped that by understanding how the IGFs and their binding proteins interact, we will be able to offer more effective therapies and develop preventive strategies for illnesses characterized by alterations in the IGFs and their binding proteins, such as diabetes mellitus.

MeSH terms: endocrinology; metabolism; endothelium; IGFs; IGF binding proteins.



PRINCIPAL INVESTIGATOR: Gail A. Bishop, Ph.D.

PROJECT TITLE:

Regulation of B Lymphocyte Function and B cell Transformation

### **SUMMARY:**

My laboratory studies the regulation of B lymphocytes, the white blood cell which produces antibodies. In addition to their critical role in antibody production, B lymphocytes serve to make foreign substances recognizable to the

other major type of lymphocyte, T cells, which mediate cellular immunity. The process of B cell activation must be very precisely regulated, as production of antibodies to normal "self" components can lead to autoimmune diseases such as rheumatoid arthritis, SLE, and diabetes. Uncontrolled B cell activation can also lead to the development of lymphomas and leukemias. The focus of my research is B cell membrane molecules which transmit regulatory signals to the inside of the cell to control its activation. One of the major molecules we study is called CD40, and this receptor delivers signals critical to antibody production, the development of the recall response (which is mimicked by vaccination), and the ability of B cells to stimulate T cells. People with defects in the CD40 signaling pathway suffer from an immunodeficiency disease which results in susceptibility to recurrent infections and decreased lifespan. Interestingly, a viral protein produced by the Epstein-Barr virus mimics the CD40 signaling pathway in B cells, but in an uncontrolled fashion. This protein, called LMP1, is necessary for the development of EBVassociated lymphomas. As >90% of the world population is latently infected with EBV by adulthood, this malignancy is a major medical complication in patients whose immune systems are suppressed by HIV infection, or treatment with post-transplant anti-rejection drugs. We are in the process of learning the details of how the LMP1 abnormal signaling pathway differs from the normal CD40 signaling pathway. It is hoped that our findings will be applicable to design of more effective therapy for B cell lymphoma and other immunological disorders.

MeSH terms: immunology; rheumatology; molecular biology; antibody response; B cells; autoimmunity; virology; lymphoma; transmembrane signaling.



PRINCIPAL INVESTIGATOR:

Bradley E. Britigan, M.D.

PROJECT TITLE:

Promotion of Hydroxyl Radicalmediated Tissue Injury by Pseudomonas Secretory Products

#### **SUMMARY:**

The bacterial species *Pseudomonas aeruginosa* is a common cause of hospital-acquired pneumonia which, even with treatment, may have a mortality that approaches 70%. This organism also causes long-term lung infections in cystic fibrosis patients. This process is responsible for most of the deaths in cystic fibrosis. Much of the problem in Pseudomonas lung infections is that they are associated with marked destruction of lung tissues. The exact mechanisms responsible for this remain largely unknown. Local and blood borne white blood cells (phagocytes) attack the Pseudomonas in the lung in an attempt to eradicate it. The microbicidal activity of phagocytes is linked to their ability to convert oxygen to other oxygen species (superoxide  $[O_2^-]$  and hydrogen peroxide  $[H_2O_2]$ ) which are toxic for bacteria. However, this same process may also damage local tissues. In the presence of some forms of iron, O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub> will react to form the extremely toxic hydroxyl radical (OH), which can damage the lung. Phagocytes do not contain iron in a form capable of allowing OH to be formed. Recent work from our laboratory has suggested that three compounds actively secreted by Pseudomonas aeruginosa may alone, or in conjunction with neutrophil (PMN)-derived  ${}^{\bullet}O_2^{-}/H_2O_2$ , lead t  ${}^{\bullet}OH$  generation and a subsequent tissue injury. These Pseudomonas aeruginosa-derived products are: pyochelin, a Pseudomonas aeruginosa siderophore (iron-chelator) which Pseudomonas aeruginosa employs to acquired microenvironmental iron and which we found is an effective OH catalyst; pseudomonas elastase, a Pseudomonas aeruginosa protease which we found cleaves the human iron-binding protein transferrin to form new iron chelates capable of catalyzing OH production; and pyocyanin, a Pseudomonas aeruginosa product which can undergo eukaryotic cell-mediated aerobic redox cycling which results in the generation of O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub>. The goal of the work proposed is to investigate the potential for these Pseudomonas

aeruginosa -derived products to contribute to tissue injury by promoting toxic oxidant formation. The ability of these compounds, alone or in combination with human neutrophils, to damage lung cells thought to be injured during the course of Pseudomonas lung infections will be examined. The mechanisms involved in this injury will be ascertained. Finally, the possibility that these compounds may induce additional changes in functional aspects of these cells that could further promote inflammatory injury to the lung will be explored. It is hoped that these studies will provide new insight into the mechanism of Pseudomonas lung dis ease thereby leading to new treatments.

MeSH terms: pseudomonas; iron; siderophore; neutrophils; hydroxyl radical; pyocyanin; transferrin; elastase; lung; infectious diseases.



### PRINCIPAL INVESTIGATOR: Bradley E. Britigan, M.D.

### PROJECT TITLE:

Biology of Macrophage Transferrin-independent Fe Uptake

### **SUMMARY:**

Iron is a metal that is required by nearly all cells for the growth and function. However, if cells contain too much iron they can be damaged through the ability of the iron to react with various forms of oxygen to produce toxic molecules (free radicals). In addition, restricting the availability of free iron in the body is felt to protect from infection. Bacteria and other microorganisms also require iron to grow. Therefore if the body has too much iron available it is likely to be more susceptible to infection. For these various reasons the availability of iron (storage and transport) is tightly regulated. Although most iron outside of cells, such as in the blood, is tightly bound to proteins (e.g.

transferrin) which specifically function to transport and store iron safely. Most of our information about iron metabolism in cells centers around how cells utilize iron bound to transferrin. However, in addition to protein-bound iron there is some iron outside of cells which is bound to small molecules such as citrate. Our laboratory has obtained evidence that macrophages, a type of white blood cell known to play an important role in iron metabolism, have a unique way to obtain iron from these small molecules. The work that we are doing is designed to identify how this novel iron uptake mechanism works and what determines how active or inactive it is. We believe that this system may be an important mechanism of regulating iron availability in the body and that it therefore is intimately linked to protecting the host from infection and free radical damage.

MeSH terms: iron; macrophages; transferrin; gallium; hydroxyl radical; phagocyte.



PRINCIPAL INVESTIGATOR:

Joseph A. Buckwalter, M.D.

### PROJECT TITLE:

Alterations in Cartilage Extracellular Matrix and the Etiology Of OA

### **SUMMARY:**

Osteoarthritis is one of the most common and disabling conditions for middle aged and older individuals. It is particularly common in the patients cared for by the VA Medical Centers. Recent work in our laboratory strongly suggests that certain alterations in the articular cartilage, the tissue that forms the bearing surface of joints, increase the risk of developing osteoarthritis. We are conducting research to define the exact nature of these alterations so that we can develop better strategies to slow the progression of osteoarthritis and ideally possibly even

prevent or reverse the course of this disabling condition. Ultimately therapies based on better understanding of alterations in articular cartilage and the role of these alterations in the development of osteoarthritis could help tens of millions on patients.

MeSH terms: osteoarthritis; articular cartilage; extracellular matrix.



PRINCIPAL INVESTIGATOR: John J. Callaghan, M.D.

PROJECT TITLE:

Biomechanics of Total Hip Dislocation

### **SUMMARY:**

Dislocation is a very troublesome complication of total hip replacement, affecting between 2 and 11 % of all patients receiving first-time hip implants, and between 4 and 25% of patients receiving a replacements of a failed hip implant. Approximately 200,000 total hip replacements are performed each year in the U.S. (most frequently when the natural hip has become intolerably painful due to arthritis), and involve implants which are essentially ball-in-hemispherical-socket mechanisms. The term "dislocation" refers to the spherical metal (femoral head) portion of the implant escaping from its hemispherical plastic (acetabular) containment. This usually occurs due to the patient performing a cautioned-against maneuver such as leg crossing while seated, or stooping low to pick up an object from the floor. Whether or not a given prosthesis actually happens to dislocate due to a given motion challenge depends upon numerous mechanical factors, especially the design of the prosthesis and how the surgeon had positioned the implant components with respect to the individual bones of the patient's hip. We have been studying the biomechanics of the dislocation process, based on measurements of human motions during

dislocation-prone activities, combined with computer modeling (finite element analysis) and laboratory bench-top measurements. The computer modeling is particularly helpful, since it allows us to identify which individual prosthesis design factors and which aspects of the implant's positioning at surgery are most influential in pre-disposing toward dislocation. And, this approach allows us to quantify which plausible improvements are actually most helpful for increasing the mechanical resistance to dislocation.

MeSH terms: biomechanics; hip prosthesis; hip dislocation.



PRINCIPAL INVESTIGATOR:

Mark W. Chapleau, Ph.D.

### PROJECT TITLE:

Baroreflex and Autonomic Dysfunction in Atherosclerosis and Hypertension

### **SUMMARY:**

The autonomic nervous system is a very important regulator of blood pressure, heart rate and overall cardiovascular function. The baroreceptor reflex is a mechanism by which the nervous system senses changes in blood pressure and elicits adjustments in the circulation that attempt to keep blood pressure normal. It is known that the autonomic nervous system and the baroreceptor reflex are altered in patients with cardiovas cular disease. This impairment in cardiovascular regulation may lead to dangerous decreases and increases in blood pressure and increase the risk of heart attacks, strokes and irregular heartbeats. The goal of my research program is to determine the mechanisms responsible for causing abnormal neural regulation of cardiovascular function in patients with cardiovascular disease, specifically atherosclerosis which involves fat buildup in

arteries and hypertension (high blood pressure). Human patients often have coexisting atherosclerosis and hypertension. There have been many studies of cardiovascular regulation in animal models of hypertension, very few studies in atherosclerotic animals, and no studies in an animal model with atherosclerosis and hypertension. My research tests the new idea that the combination of atherosclerosis and hypertension will cause greater dysfunction in cardiovascular regulation than that observed in the individual disease states. To test this idea we study a genetic strain of mice that develops atherosclerosis without hypertension, a genetic strain of mice that develops hypertension in the absence of atherosclerosis, and mice genetically altered to cause both atherosclerosis and hypertension, as well as control healthy mice. I have chosen specific types of genetically modified mice in order to let me test the roles of two important factors in causing the autonomic and baroreflex dysfunction. I believe that a chemical factor called angiotensin and a group of substances referred to as reactive oxidant species or free radicals may be important causes of the alterations in blood pressure regulation, particularly when these factors react together in atherosclerotic hypertensive subjects. We study baroreceptor reflex sensitivity and other blood pressure regulating mechanisms in the genetically modified mice by measuring blood pressure and heart rate in conscious mice and directly measuring the activity of baroreceptor nerves that sense changes in blood pressure and the activity of nerves regulating heart and blood vessel function in anesthetized mice. We use drugs that inhibit the production of angiotensin and oxygen free radicals to prove the importance of these mechanisms and to restore blood pressure regulation to normal in the diseased animals. Furthermore, we use special viruses that have been developed to allow transfer of genes to specific sites important in the neural regulation of the cardiovascular system. Using this approach we hope to improve baroreflex sensitivity in the atherosclerotic hypertensive mice and demonstrate the beneficial consequences on blood pressure regulation. We believe that the results of our research will advance our knowledge of basic mechanisms that cause abnormal blood pressure regulation in patients suffering from atherosclerosis and hypertension and may lead to development of new therapies for patients.

MeSH terms: blood pressure; pressoreceptors; carotid sinus; atherosclerosis; hypertension; sympathetic nervous system; cardiovascular disorders.



PRINCIPAL INVESTIGATOR:

Michael B. Cohen, M.D.

PROJECT TITLE:

Cell Death in Human Prostate
Cancer

### **SUMMARY:**

The laboratory utilizes immunologic and molecular techniques to investigate prostate cancer. The major ongoing focus in the laboratory is to examine members of the TNF receptor family in apoptosis (programmed cell death). We are studying three members of this family (Fas, TNF-alpha & TRAIL). We have identified that certain prostate cancer cell lines are resistant to apoptosis induction by one or more of these ligands. Studies are ongoing to understand the mechanism(s) of resistance to these signaling pathways. In addition, the laboratory has had a long standing interest in the pre-neoplastic lesions of the prostate and their progression to invasive and metastatic disease. We are studying several animal models to investigate biomarkers (intermediate end points) during this complex process.

MeSH terms: prostatic neoplasms; apoptosis; programmed cell death; Fas; TNF-alpha; TRAIL.



PRINCIPAL INVESTIGATOR: Brian L. Cook, D.O.

PROJECT TITLE:
A Prospective Outcome Study in
Alcoholism

### **SUMMARY:**

This study is a follow-up investigation of 445 veterans who were admitted to the psychiatric unit at the Iowa City VAMC and participated in an interview study in 1975-1976. Nearly half of these veterans were diagnosed with alcoholrelated problems at that time. This study is being conducted to re-interview all of these individuals to assess their long-term outcome in terms of drinking behavior, mortality, and medical morbidity. The research project is also trying to evaluate whether individuals with alcohol problems that are accompanied by other (comorbid) psychiatric disorders have different outcomes than those without co-morbid psychiatric disorders. We are still attempting to relocate and interview all veterans still living. Analysis of mortality rates has been completed and it demonstrates dramatic and interesting findings. The alcoholics on average have died over 20 years earlier than would be predicted when they are compared to age and sex matched non-alcoholics who were born during the same years as the veterans being studied. Alcoholics with antisocial personality disorder appear to have the best prognosis in terms of longevity, while alcoholics with and without other psychiatric disorders are about the same. This information provides interesting implications for treatment. Alcoholics with antisocial personality disorder are often thought to have poor prognosis based on short-term follow-up studies of drinking behavior. This study demonstrates that this group may actually do better in the longterm, presumably because as their disruptive personality traits diminish with age, so does their drinking. These antisocials also have a pattern of drinking that is more episodic, and this may play a role in their lowered mortality rates. These issues all are being studied in more detail, and will hopefully result in information that could improve treatment for alcoholics in general.

MeSH Terms: alcoholism; cohort studies; mortality; outcome assessment.



### PROJECT TITLE:

Cellular Immune Function and Phenotype In Multiple Myeloma

Robert T. Cook, M.D., Ph.D.

### **SUMMARY:**

Patients with multiple myeloma experience progressive immune deficiency and increased rates of serious infectious disease, both as part of the natural disease progression and variably as a result of cytotoxic therapy. Both B cell (antibody producing) dysfunction and CD8+ (suppressor/cytotoxic lymphocyte) or NK (natural killer) cell alterations are described in these patients, and are thought to be involved in the progressive immune deficiency. Our longrange goal is to understand the cellular immune mechanisms responsible for the immune deficiency/immune suppression, so that schemes to counteract the deficiency can be tested.

We are investigating in detail the changes occurring in the non-malignant peripheral blood lymphocyte subpopulations in myeloma patients to relate the cellular changes to the development of immunosuppression. We have examined the peripheral blood lymphocytes of several patients by three and four colour flow cytometry. The results show major shifts in T cell phenotype to a highly activated profile. Both CD8hi and CD4+ T cells have greatly increased expression of CD57, an activation-related molecule usually associated in lymphocytes with NK cells.

Current work is directed toward identification of the factors involved in the abnormal activation of the T cell subsets. Special attention is directed toward the expression of CD40 ligand, an important regulator of antibody synthesis. These studies may lead to improved understanding of the control of antibodies, which in turn will improve our understanding of how to treat persons with immunodeficiency states.

MeSH Terms: myeloma; T lymphocyte; immunodeficiency.



### PRINCIPAL INVESTIGATOR:

Robert T. Cook, M.D., Ph.D.

### PROJECT TITLE:

Alcoholism: Modulation and Function of Lymphocyte Subsets (Clinical Immunology of Alcoholism)

### **SUMMARY:**

We have studied the peripheral blood lymphocytes of alcoholics by flow cytometry to determine whether changes in cellular immune markers are present. In these and in longer-term studies, the objectives have been to correlate any changes in immunophenotype with the clinical state of the patient with regard to either immune suppression or immune activation in conditions commonly seen in the alcoholic such as infectious diseases or alcoholic hepatitis, respectively.

We have demonstrated in alcoholics without active liver disease {1} a significant increase in activated CD8 cells (J. Clin. Immunol. 11 #5, 1991); {2} substantial imbalance in the fine subsets of both CD4 and CD8 lymphocytes (Alcoholism: Clinical and Experimental Research 18 #1, 1994); {3} an increase in many patients of a normally minor lymphocyte subset, the

CD8+CD57+ subset (Alcoholism: Clinical and Experimental Research 19 #3, 555-563, 1995); {4} a decrease in the percentage of lineage negative lymphocytes which are CD56+; {5} a substantial loss of the CD5+ B cell subset. Detailed examination of the fine T lymphocyte subsets reveals several changes in pattern. There is decreased expression of L-selectin (lymphocyte homing receptor), and a decreased percentage of both CD4 and CD8 cells which are CD45RA. CD8hi lymphocytes have increased expression of the adhesion glycoprotein CDIIb which is accompanied by a decrease in the expression of L-selectin. Reversal of the changes in T-cell immunophenotype begins during the first week after alcohol withdrawal, but it appears that the rate of return toward normal varies widely for different cellular markers. The overall pattern of T-cell fine subset changes raises the possibility that these patients are developing an increase in T-cell cytotoxic potential and a decrease in regulatory cells while drinking.

We are additionally characterizing the peripheral blood lymphocytes of alcoholics by phenotype and by functional studies. Phenotypic studies are by three and four-color flow cytometric methods, with special attention to CD3 + CD8hiCD57 +, CD3 + CD8hiCD57--and CD3--CD19--subsets. Functional studies focus on the killing activity and the regulatory activity of both whole lymphocyte fraction and subsets obtained by sorting. Functional studies include: {1} measurement of fresh and lymphokine activated killing activity by natural killer and MHC-nonrestricted T killer cells; {2} measurement of killing activity and its augmentation in the expanded CD3--CD19--CD56- cell subfractions; {3} the capacity for development of MHC-restricted Tcell cytotoxicity in an allo-driven system; and {4} the ability of putative regulatory T-cell subsets to inhibit B-cell proliferation and IgG production in vitro.

Recently, we have discovered up-regulation of a T-cell molecule, CD40 ligand, in chronic alcoholics. This molecule is known to be critical in the regulation of antibody synthesis, and its continuing study is one of our major goals.

MeSH Terms: alcoholism; Tlymphocyte; natural killer cells.



### PRINCIPAL INVESTIGATOR: Robert T. Cook, M.D., Ph.D.

### PROJECT TITLE:

**Alcohol Effect on HIV Infectivity** and Cellular Responses

### **SUMMARY:**

HIV infection has become one of the leading causes of death and morbidity in the young adult age group in the U.S. and worldwide. Understanding predisposing factors to HIV infection is of great clinical relevance, especially one so widespread as casual alcohol ingestion. As a result of recent published work, the question has been raised as to whether casual alcohol exposure, as in ordinary social drinking, results in increased biologic susceptibility to HIV infection as distinct merely from an increase in risk-taking behavior. Therefore, it is the objective of this study to determine whether the isolated white blood cells of normal HIV-seronegative volunteers are more susceptible to in vitro (in the test tube) HIV infection after the volunteer drinks alcohol as compared with before drinking alcohol.

Twelve normal volunteers who are HIV seronegative by standard HIV test were given alcohol (1g/kg) in carefully measured doses under controlled conditions in the Clinical Research Center at UIHC. Blood was drawn before and after at several intervals for testing of immune function parameters including immunophenotyping and several activity assays, and for evaluation of susceptibility to in vitro infection by standard test strains of the HIV virus.

Administration of 1g/kg of ethanol to the 12 volunteers in 30 minutes did not result in any serious reactions. All routine blood chemistries remained normal and all volunteers reported for work the following morning without difficulty. Analysis of the results did not demonstrate a clear predisposition to infection of the isolated lymphocytes after alcohol ingestion at this level.

The data set obtained continues to provide a valuable comparison with the chronic alcoholics under study in our laboratory.

MeSH Terms: HIV; AIDS; alcoholism; T lymphocyte.

molecules that are involved in CpG DNA recognition or subsequent activation events.

MeSH terms: interleukin-12; interferongamma, recombinant; DNA, bacterial.



### PROJECT TITLE:

Bacterial DNA Induction in Macrophage/Dendritic Cell IL-12 Production

### **SUMMARY:**

Bacterial DNA (bDNA) is known to be a strong mediator of inflammatory responses and a potent adjuvant capable of boosting antibody and cytotoxic responses. An important way the element in bDNA induces inflammation and immune response is by induction of interleukin-12. Interleukin-12 is capable of augmenting a number of other proinflammatory cytokines such as interferon-γ tumor factor-α. Work by others has established that the recognition of bacterial DNA depends upon the presence of internal CpG motifs. The precise mechanism of recognition remains unknown. A major emphasis of this project is to use a genetic approach to identify critical molecules that are involved in the recognition of CpG DNA and molecules that may be involved in subsequent signal transduction events. We have established a cell line in which green fluorescent protein is driven by the IL-12 p40 promoter. We have mutated this cell line, and we have isolated mutants which respond to lipopolysaccharide but not to CpG DNA. Work in progress involves using a retroviral gene rescue technique to establish the identity of

### PRINCIPAL INVESTIGATOR: Joseph J. Cullen. M.D

### PROJECT TITLE:

Control of Esophageal Motor Function: Roles of Antioxidant Enzymes and Superoxide

### SUMMARY:

Reflux esophagitis is a common problem that produces heartburn, esophageal ulcerations, bleeding, strictures, Barrett's epithelium (a precancerous lesion), and esophageal carcinoma; all of which cause significant morbidity and mortality in VA patients. It is commonly associated with esophageal motor abnormalities that perpetuate the inflammation by allowing more frequent gastroesophageal reflux and by decreasing the clearance of refluxate from the esophagus. Esophageal spasm, the most disconcerting of these motor disorders, produces chest pain indistinguishable from angina. Understanding the relationhip between esophagitis and altered esophageal motor function may help in the management of both. Lower esophaeal sphincter (LES) relaxation and the sequencing of peristalsis in the smooth muscle esophagus depend upon the release of nitric oxide (NO) from intrinsic esophageal nerves. Inhibiting NO neurotransmission disrupts esophageal peristalsis and produces esophageal spasm. The fact that motor abnormalities caused by disrupting NO

neurotransmission are like those seen with reflux esophagitis suggests that inflammation may disrupt the NO signaling system. Inflammatory states have the generation of oxygen-derived free radicals (O<sub>2</sub>), and the induction of NO synthase (NOS) as common features. The generation of O<sub>2</sub> and NO may be important pathophysiological mechanisms by which esophageal motor function is altered in these inflammatory states. Superoxide rapidly oxidizes NO to form peroxynitrite (ONOO); thereby, terminating the physiological actions of NO. Thus, O<sub>2</sub> may scavenge NO released from esophageal nerves and disrupt esophageal motor function. Peroxynitrite, in turn, alters cellular proteins, providing a potential way by which cellular signaling systems may be disrupted. Thus, the esophageal motor changes that accompany esophageal inflammation may be the result of O<sub>2</sub> scavenging NO and/or the production of ONOO. We use the opossum esophagus - a model of esophageal motor function that is anatomically and physiologically like that of the human - to explore the roles that O<sub>2</sub>, ONOO, and the antioxidant systems, play in modulating esophageal neuromuscular function in the normal and inflamed esophagus. Our studies will test 6 general hypotheses: 1) NOmediated motor functions of the esophagus can be antagonized by  $O_2$  and that the antioxidant enzyme superoxide dismutase (SOD) can inhibit this effect; 2) the antioxidant enzymes SOD and catalase (CAT) are localized to the muscularis propria of the esophagus, specifically esophageal nerves or muscles; 3) peroxynitrite alters esophageal motor function; 4)  $O_2$ , and/or peroxynitrite modulate the actions of NO by interfering with certain elements of the NO signal transduction system; 5) esophagitis alters i) superoxide generations, ii) the NO generating system, and iii) the antioxidant enzyme system; 6) oxidative stress and/or peroxynitrite generation play a role in the esophageal motor abnormalities associated with esophagitis. These studies will shed new light on the pathophysiological mechanisms by which inflammation alters the motor function of the esophagus, and they will lay a solid groundwork for future work exploring treatments for disorders like esophageal spasm and noncardiac chest pain.

MeSH terms: nitric oxide; muscle, smooth; esophagus; esophageal motility disorders; gastroenterology; superoxide; esophagitis.

PRINCIPAL INVESTIGATOR: Joseph J. Cullen, M.D.

PROJECT TITLE:

The Role of Free Radicals in
Biliary Tract Motility

### **SUMMARY:**

Biliary tract disease, including gallbladder and sphincter of oddi dysfunction, is a common problem. Biliary tract disease affects over 20 million Americans with an increasing incidence with advancing age. More that 600,000 cholecystectomies are performed annually in the United States, making this one of the most common surgical problems. Considerable evidence suggests that altered biliary tract motility may play an important role in the majority of gallbladder conditions. Both clinical and experimental studies demonstrate that altered gallbladder motility is a mechanism in the pathogenesis of pigment and cholesterol gallstones, acute acalculous cholecystitis, and chronic acalculous cholecystitis. Inflammatory disorders impair biliary tract motility. All inflammatory states have the generation of oxygen-derived free radicals as a common feature. Our laboratory has demonstrated that oxygen radical generation is an important pathophysiological mechanism by which biliary tract motor function is altered. Oxygen radical rapidly oxidizes nitric oxide in the biliary tract to form peroxynitrite, thereby terminating the actions of nitric oxide. Peroxynitrite in turn can be protonated to form cytotoxic radicals. Thus, the biliary tract motor changes that accompany inflammation may be the result of oxygen radical scavenging nitric oxide or the production of peroxynitrite. The logical extension of our previous work is now to address the mechanisms by which free radicals impair biliary tract neuromuscular function during the inflammatory conditions that accompany calculous and acalculous cholecystitis. Insight into these mechanisms will aid our understanding of the

inflammatory conditions of the biliary tract, which may lead to potential targets for treatment.

MeSH terms: cholecystitis; biliary tract diseases; surgery.



PRINCIPAL INVESTIGATOR:

Kevin C. Dellsperger, M.D.

PROJECT TITLE:

Mechanisms of Coronary Microvascular Regulation

### **SUMMARY:**

Endothelial cells lining small arteries and arterioles of the heart release several substances that determine tone of the vessel and therefore blood flow to the heart. Previously characterized mediators of endothelium-dependent vasodilation are nitric oxide and prostaglandins. Another factor, which causes vascular relaxation by activating potassium channels, has been termed endothelium-dependent hyperpolarizing factor (EDHF). EDHF may account for the majority of coronary microvascular dilation. Recently, our laboratory has shown that inhibitors of cytochrome P<sub>450</sub> enzymes block acetylcholine induced vasodilation in the coronary microcirculation. Epoxyeicosatrienoic acids (EETs) are the metabolic products of arachidonic acid through the cytochrome P<sub>450</sub> pathway. Our hypotheses are that EETs may be an EDHF and are important in relaxation in the coronary microcirculation. We will specifically evaluate small coronary vessels approximately 100? m. the size of a human hair. These microvessels are the primary areas that control blood flow to the heart. Studies will be performed using in vivo analysis of the coronary microcirculation in a beating left ventricular preparation. In vitro studies including isolated coronary microvessel preparations, measurements of membrane potential and patch clamp methods of isolated coronary microvascular smooth muscle cells to identify potassium channel activity will also be performed. These studies will help to identify mechanisms responsible for microvascular control of the coronary circulation and provide information concerning potential therapies for vascular diseases.

MeSH terms: cytochrome P-450; endotheliumderived relaxing factor; coronary circulation.



PRINCIPAL INVESTIGATOR:

Kevin C. Dellsperger, M.D.

PROJECT TITLE:

**Mechanisms of Diabetes Endothelium Dysfunction** 

### **SUMMARY:**

Recent epidemiological studies indicate that diabetes increases the risk of developing cardiovascular disease 2 to 6 fold. This may, in part, be due to abnormalities in function of blood vessels in patients with diabetes. Our objective is to determine vascular dysfunction associated with diabetes and potential therapies to improve heart function and consequently the quality of life of diabetic patients. We will specifically evaluate the small coronary vessels that are approximately 100? m, the size of a human hair. These microvessels are the primary areas that control blood flow to the heart. We will use highly sophisticated methods that are possible only at two centers in the world. We will study microvessels in intact animals with diabetes as well as in isolated microvessels from the hearts of diabetic animals. We will examine adverse vascular effects from long-term (5-year) diabetic

animals. Other studies will evaluate insulin and insulin-like growth factor dilation in coronary vessels of different sizes. Some animals will be given various treatments (such as antioxidants and vitamin E) to evaluate whether the abnormal function in diabetes is corrected. We hope that these findings will provide a sound experimental base from which to generate clinical trials regarding the role of antioxidants in diabetic patients within our Diabetes Research Center during the final year of funding.

MeSH terms: diabetes mellitus; endothelium; coronary microcirculation.

respiratory disease, inflammatory bowel disease, and vaccine development.

MeSH terms: immunity: epithelial cells: IgA:

pathogens. Among the many areas that would

benefit from this knowledge are the areas of

MeSH terms: immunity; epithelial cells; IgA; interleukin-r; interferon-gamma; mucosal.



### PRINCIPAL INVESTIGATOR: G.M. Denning, Ph.D.

### PROJECT TITLE:

Cytokine-dependent Regulation of the Polymeric Iga Receptor in Human Intestinal Epithelial Cells

### **SUMMARY:**

Infectious diseases continue to present a serious threat to our veteran population. These diseases are caused by numerous pathogens, including viruses, bacteria, and parasites. The sites of infection for many of these pathogens are the surfaces that line the airway, intestine, and genitourinary tracts (referred to as mucosal surfaces). The body protects itself against these mucosal pathogens by producing antibodies that must then be transported to the mucosal surface where the pathogen resides. The protein that transports these antibodies is called the polymeric immunoglobulin receptor. Our laboratory is interested in understanding how transport by this receptor is regulated both under normal conditions, as well as during infection. To address these questions, we use numerous techniques in cell biology and molecular biology. Our studies should provide basic knowledge about the mucosal immune response to these

PRINCIPAL INVESTIGATOR: G.M. Denning, Ph.D.

### PROJECT TITLE:

Mechanisms by Which Pseudomonas Aeruginosa Secretory Factors Contribute to Airway Disease

### **SUMMARY:**

Pseudomonas aeruginosa is the major bacterium that causes life-threatening lung disease in immuno-compromised hospital patients. Over 70% of those infected die, even with appropriate antibiotic treatment. Pseudomonas secretes many factors that affect lung function. Studies by our laboratory are designed to identify bacterial factors that might contribute to lung disease, determine the effect of these factors on airway cells, and identify therapeutic compounds that might protect against these effects.

MeSH terms: epithelial cells; signal transduction; interleukin-8; pyocyanine; pseudomonas aeurginosa.

### PRINCIPAL INVESTIGATOR: Gerald F. DiBona, M.D.

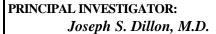
PROJECT TITLE:

Differentiated Sympathetic
Neural ControloOf the Kidney

### **SUMMARY:**

The kidney nerves travel from the nervous system (brain and spinal cord) to the kidney. The kidney nerves, by carrying information from the nervous system to the kidney, regulate the functions of the kidney. These kidney functions are the blood flow to the kidney, the formation of urine and the production of various substances by the kidney. These kidney functions are abnormal in patients with high blood pressure and in conditions where the body retains excess salt and water, such as heart, kidney and liver failure. It is the purpose of this project to understand how the kidney nerves cause these abnormal kidney functions. A better understanding of this process would enable the development of newer, more beneficial and cost effective treatments in such patients.

MeSH terms: nephrology; kidney; renal sympathetic nerves; renal function.



### PROJECT TITLE:

Development of Novel Genes for Type II Diabetes Using the GK Rat Model

### **SUMMARY:**

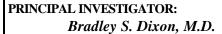
Diabetes Mellitus effects more than 10 million American citizens today. Adults with this disorder usually have a strong family history of diabetes. This suggests that genetic abnormalities are an important cause of the disease. Although rare gene defects have been found in families with very unusual forms of diabetes, attempts to define the genetic defects in the common form of adult onset diabetes have been disappointing.

Many human genetic abnormalities have first been defined in suitable animal models. The absence of a good animal model of adult onset diabetes has hampered the search for the genetic basis of this disorder. However, a recently described rat species, the GK rat, has a form of diabetes very similar to that found in human adults. This rat has insufficient insulin secretion from the pancreas and this abnormality appears to be related to a genetic mutation.

A further hurdle in defining the genetic basis of diabetes mellitus has been the difficulty of obtaining sufficient tissue from the insulin secreting cells of the pancreas to examine the genetic material. A recently described technique called polymerase chain reaction now allows us to use tiny quantities of DNA for these investigations.

We propose to compare the genetic material from diabetic GK and normal Wistar rat insulin producing cells, to determine which genes are abnormal in the GK rat. We will use the polymerase chain reaction technique to perform these investigations on tiny quantities of tissue. Once we define the abnormalities in the GK rat we will examine for these specific abnormalities in humans with adult onset diabetes. We hope that by using these novel techniques and the GK rat we can isolate the abnormal genes for human diabetes and thus suggest novel targets for drug therapy in this disorder.

MeSH terms: diabetes mellitus; genetic abnormalities.



PROJECT TITLE:

Growth Factor Regulation of the Bradykinin Receptor in Vascular Injury

#### **SUMMARY:**

Arteriosclerotic cardiovascular disease is the most common cause of morbidity and mortality in developed nations. An important component of the arterial narrowing seen with arteriosclerosis is due to proliferation of vascular smooth muscle cells. Proliferation of vascular smooth muscle cells can be stimulated by a variety of inflammatory chemical factors which are normally sequestered or quiescent in the blood stream but are activated after injury. These factors help to repair the injured wall of the blood vessel but secondarily lead to narrowing of the arterial lumen and impaired blood flow to the affected organ. The current proposal is focused on the inflammatory hormone, bradykinin and regulation of its receptors on vascular smooth muscle cells. Bradykinin is released by inflammation and clotting which occurs at the site of vascular injury. We have found that bradykinin acting via its receptors can inhibit proliferation of arterial smooth muscle cells stimulated by potent growth factors such as platelet-derived growth factor (PDGF). In addition, we have found that PDGF upregulates the expression of receptors for bradykinin on vascular smooth muscle cells. This suggests an interesting feedback loop whereby the potent blood-derived growth factor (PDGF) upregulates the expression of bradykinin receptors which can then act to inhibit or control smooth muscle cell proliferation by PDGF. This is of further interest because bradykinin is degraded by an enzyme known as angiotensin converting enzyme present on the surface of blood vessels. Drugs which inhibit angiotensin converting enzyme are one of the currently most effective agents used in the treatment of

cardiovascular and renal diseases. Inhibition of this enzyme will lead to increased levels of bradykinin that may act as a protective hormone to decrease smooth muscle cell proliferation. Further strategies to upregulate the expression of bradykinin receptors and the coupling of these receptors to inhibition of vascular smooth muscle cell proliferation may be useful in further controlling arteriosclerosis. Research in my laboratory is examining the cellular signaling pathways by which growth factors such as PDGF upregulate bradykinin receptor expression. Understanding these cellular pathways which regulate expression of these kinin receptors may open up new strategies for regulating arteriosclerosis.

MeSH terms: muscle, smooth vascular; bradykinin; angiotensin converting enzyme; growth factors; receptors; biochemistry; signal transduction; molecular biology; inflammation.



### PRINCIPAL INVESTIGATOR:

Bradley N. Doebbeling, M.D.,

MSc

PROJECT TITLE:

**Epidemiology and Control of Antimicrobial Resistance in Hospitals** 

### **SUMMARY:**

There is an unprecedented worldwide crisis related to the rapid emergence and dissemination of antimicrobial resistant organisms. Important pathogens are increasingly resistant to previously effective agents. With excessive use of broad-spectrum antimicrobials, resistance to previously effective agents has become common among microorganisms infecting hospitalized patients. Furthermore, inconsistent use of basic infection control techniques by hospital personnel has contributed importantly to the spread of resistant strains within hospitals. Concern about the transfer of resistance to

organisms with greater ability to cause severe disease than currently resistant microbes, raises the specter of frequent infections for which no effective antimicrobial treatment is known. Previous efforts to control resistance have not worked. Perhaps this is partially due to the complex series of factors within hospitals that act locally to influence the development and implementation of policies. National consensus strategic goals or actions designed to prevent the emergence and control the spread of antimicrobial resistance have been published, including specific process and outcome measures to evaluate progress towards these goals. However, the impact of these strategic goals on the prevention or control of antimicrobial resistance within hospitals has not been evaluated. The proposed observational or quasiexperimental project will describe the prevalence and secular trends of antimicrobial resistance among nosocomial pathogens over time in a national sample of VA and non-VA acute care hospitals. The study will assess the degree of implementation of specific components of recommended antimicrobial control measures at the institutional level, and determine the effectiveness of specific recommended measures in the prevention and control of emergence and spread of antimicrobial resistance. The sources of data for the study will include: provider and administrator surveys, review of laboratory, infection control and pharmacy data, review of institutional policies and reports, and review of medical records. It is expected that this study will significantly advance our understanding of those factors important in the prevention and control of antimicrobial resistance during patient care. Furthermore, these results should help inform subsequent intervention studies to optimally protect the VA patient care population and prevent adverse outcomes among VA patients and others.

MeSH terms: antimicrobial resistance; nosocomial pathogens; organizational factors; implementation; outcomes of care.



PRINCIPAL INVESTIGATOR:

Bradley N. Doebbeling, M.D.,

*MSc* 

PROJECT TITLE:

Determinants of Clinical Guideline Implementation Effectiveness

### **SUMMARY:**

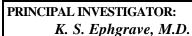
Following clinical guidelines to improve quality of medical care is affected by both a hospital's structure as well as the processes in place to implement the guidelines. Guideline implementation teams work within organizations whose structural characteristics, managerial attitudes and relationships to outside entities influence the composition and working style of the teams. Compliance with a guideline is also influenced by the characteristics of the guideline itself. This study will investigate the relationship between VHA facilities' organizational characteristics, structures of implementation teams and the processes they employ, with effective guideline implementation. We will focus primarily on how providers comply with the guidelines at the facility level. Additionally, we will examine these factors and effects across several guidelines to explore how the characteristics of the guideline itself affects implementation and compliance. This is a multimethod, quasi-experimental national study utilizing qualitative and quantitative research methods to study the process of guideline implementation within VHA hospitals over time. We will perform focus groups and structured interviews at approximately 20 VAMCs nationally representing a range of geographic, bedsize, teaching affiliation, and patient population gender and racial/ethnic distributions. Focus groups and individual interviews of primary care providers and managers at selected facilities will be performed in order to better understand the process of clinical guideline implementation and identify important barriers and facilitators. In the second phase, the focus group/interview results will be used to develop written surveys of a national sample of VAMC primary care providers and administrators. Factors of interest include implementation methods, compliance,

institutional support, availability of feedback, and new approaches to incorporating guidelines into clinical practice. Multivariate regression analyses will be used to examine relationships between organizational structural factors, processes, and outcomes of care. Our study should identify factors at the organization, provider and manager level associated with the effective implementation of VA recommended clinical practice guidelines, which may ultimately lead to delivery of higher quality patient care.

MeSH terms: guidelines; compliance; organizational factors; implementation; outcomes of care; multi-method research.

addresses the question of whether the central nervous system is necessary for the protective effects of nutrients, or whether reflexes within the stomach wall by themselves can be protective. The harmful effects of stress can be overridden within the central nervous system by the neurotransmitter calcitonin, and we plan to determine whether nutrients within the GI tract also work on the calcitonin pathways to override stress' stimulation of gastric contractions and acidity. Finally, we plan to determine whether the harmful effects of stress or the protective effects of nutrients are affected by agining.

MeSH terms: surgery; gastroenterology; gastric injury; gastric motility; stress.



PROJECT TITLE:

Acute Gastric Mucosal Injury and the Gastric Responses to Stress

### **SUMMARY:**

Serious illnesses cause physiologic stress that is associated with injury to the lining of the stomach. These major stresses sometimes result in bleeding from the stomach lining in seriously ill patients. In laboratory animals, stress-induced gastric injury is linked to changes in the central nervous system, which cause giant contractions and increase acid secretion. We have been investigating how food substances block the harmful effects of stress. Our ongoing work

PRINCIPAL INVESTIGATOR:

Bernard Fallon, M.D.

PROJECT TITLE:

Prostate Intervention vs. Observation Trial [PIVOT]

### **SUMMARY:**

Prostate cancer is an extremely common cancer in men. The incidence seems to be rising. There is some dispute with regards to the best form of treatment. The standard treatment for cancer which is localized to the prostate with no evidence of spread to other areas is surgical removal of the prostate or radical prostatectomy. Some studies suggest, however, that radical prostatectomy may not be necessary. These studies suggest that observation of the patient

with treatment instituted only if symptoms should arise may be associated with good longterm survival, which is comparable to the survival following radical prostatectomy. The PIVOT study is designed to compare these two methods of management of prostate cancer. That is radical prostatectomy vs. observation and symptomatic treatment. This is a multi-institutional study intended to result in a group of approximately 1200 men who will undergo radical prostatectomy or will have treatment by observation and intervention only when symptoms occur. The patients will be randomized to one of the two groups. The cancer must be localized at the time of diagnosis to the prostate only with no evidence of spread. The patient must be less than 75 years old. They will be followed at intervals of six months with blood tests and occasional bone scans. Data regarding the two methods of therapy will be gathered with the passage of time. Final data will not be conclusive until at least 12 years have passed.

MeSH terms: prostate cancer; radical prostatectomy; symptomatic treatment.



PROJECT TITLE:

Petidergic Mechanisms in the Nucleus of the Solitary Tracts

### **SUMMARY:**

This study, originally aimed towards studying the effects of neuropeptides on the central nervous system in the regulation of arterial pressure and sympathetic drive, has been expanded to address the role of these central neural mechanisms in the evolution of congestive heart failure. In heart failure, the production of circulating peptides - including angiotensin II (ANGII) and atrial natriuretic factor (ANF) - is strikingly increased. In addition to their peripheral effects, which are well studied in heart

failure, these peptides have central neural effects that have not been systematically examined in the heart failure setting. ANGII and ANF can access brain tissue only in certain regions, called circumventricular organs (CVOs), which lack a blood-brain barrier. In the forebrain, these CVOs are in close proximity to, and at least in normal animals have a significant influence upon, critical brain regions regulating extracellular fluid volume and sympathetic nerve activity. This study focuses on the central influences of ANGII and ANF, acting at CVOs in the forebrain, on the volume retention and the augmented sympathetic drive that characterize advanced heart failure.

Studies are performed in rats with heart failure induced by coronary occlusion, simulating the most common cause of heart failure in humans. Studies in conscious rats include metabolic cage measurements of sodium and water balance, blood sampling to measure circulating peptides, and sympathetic nerve recordings. Studies in anesthetized rats are directed towards defining the central pathways and neurotransmitter mechanisms mediating the central responses to circulating peptides. It is anticipated that this work will focus attention on the contribution of central nervous system mechanisms in heart failure, and may ultimately help identify new targets for therapeutic intervention.

MeSH terms: congestive heart failure; angiotensin; neuropeptides.

PRINCIPAL INVESTIGATOR: Elizabeth H. Field, M.D.

PROJECT TITLE:
CD4 Regulatory Cells in
Immune Tolerance

### **SUMMARY:**

Immunologic tolerance is a critical property of the immune system that allows the body to rid itself of pathogens (viruses, bacteria or cancerous

cells) while protecting the body's normal cells and organs. The treatment of many diseases, including cancer and autoimmunity, requires an understanding of how the body achieves immunologic tolerance. For example, the successful transplantation of an unrelated organ or bone marrow requires that the recipient develop immune tolerance to the donor tissue. Because we cannot easily examine the human immune system, we have studied a mouse model of transplantation tolerance in order to understand the mechanisms of how the immune system achieves tolerance. We generate tolerant mice by infusing neonatal mice with lymphoid cells from adult mice of an unrelated strain. Tolerance mice develop donor cell microchimerism and accept skin grafts from the donor strain. The cytokine IL-4 plays a critical role in the induction of tolerance, and the development of tolerance is associated with immunoredirection of effector T cell populations. Tolerant mice fail to develop anti-donor CD8 cell immunity and preferentially expand anti-donor Th2 CD4 cells over Th1 CD4 cells. In contrast. non-tolerant mice that reject grafts contain antidonor CD8 effector cells and expand Th1 CD4 cells over Th2 CD4 cells. Tolerant mice also contain a CD4+CD25+ regulatory population that may function to maintain tolerance by keeping anti-donor CD8 effector cells in a state of anergy. Although the phenotype of the regulatory cells has not been completely established, the cells secrete TGF-beta in response to antigen (tolerogen) and do not appear to be conventional Th2 CD4 cells. Understanding how CD4+CD25+ cells regulate the development of alloreactive CD4 and CD8 cells will aid in the design of therapies that establish a new state of immune tolerance. This kind of therapy would benefit patients with autoimmune diseases, such as diabetes or rheumatoid arthritis, or transplant patients.

MeSH terms: immunology; tolerance; CD4 cell; cytokines.

<del>----</del>

PRINCIPAL INVESTIGATOR:

F. Jeffrey Field, M.D.

PROJECT TITLE:

Regulation of Intestinal Cholesterol Metabolism

### **SUMMARY:**

The purpose of this project is to investigate what factors in our diet and in our blood regulate cholesterol synthesis and absorption by the small intestine. The factors we are examining include different fats, cholesterol, plant sterols, oxygenated sterols, and lipoproteins to see what effects they might have on cholesterol metabolism in intestine. Our laboratory is studying this problem using several different approaches. We use cell culture and animal models to investigate cholesterol metabolism at the organ, cellular, and molecular level. We will take our observations and apply them to humans to address what can be done to alter the amount of cholesterol that enters our body daily. In the aging veteran population, atherosclerotic heart disease is a major cause of death and morbidity. A high cholesterol level in the blood is a recognized risk factor for coronary heart disease. The small intestine plays a critical role in regulating how much cholesterol gets into our blood. Thus, our studies are directed at finding new ways to decrease cholesterol synthesis and absorption by the small intestine to lower blood cholesterol levels. If this goal is accomplished, then the study will have an impact on treatment strategies for hypercholesterolemia, and hence, coronary artery disease. These interventions could potentially save the lives of our veteran population and decrease existing morbidity.

MeSH terms: gastroenterology; arteriosclerosis; cholesterol; intestine.

•

PRINCIPAL INVESTIGATOR:
Sue E. Gardner, Ph.D., RN

PROJECT TITLE:
The validity of the Clinical Signs and Symptoms Used to Identify **Localized Chronic Wound** Infection

#### **SUMMARY:**

The timely diagnosis and treatment of chronic wound (e.g., bedsores, venous ulcers, diabetic ulcers) infection is important because infection can lead to life-threatening complications. Unfortunately, the classical signs of infection (i.e., pain, erythema, heat, and purulent exudate) may not be expressed in chronic wounds despite high levels of microorganisms. Signs of infection that are specific to secondary wounds (i.e., serous exudate, delayed healing, discoloration of granulation tissue, friable granulation tissue, pocketing at the base of the wound, foul odor, and wound breakdown) may provide a better indication of chronic wound infection. This study examines how well the signs and symptoms in each of these two groups were associated with chronic wound infection. Thirtysix subjects with chronic wounds were assessed for these 12 signs and symptoms of infection. The wounds were then cultured using tissue biopsies obtained from the center of the wound. Of the 36 wounds, 11 (31%) were infected based on culture findings. The findings of this study suggest that the clinical signs and symptoms of chronic wound infection are useful for clinical decision-making, with the exception of pocketing of the wound base. The signs specific to chronic wounds appear to be better indicators of chronic wound infection than the classical signs and symptoms. This study needs to be replicated with a larger sample in order to explore the influence of advancing age, diabetes, and type of chronic wound on the accuracy of specific signs and symptoms.

MeSH terms: wound healing; YYl; wound infection; infection



PRINCIPAL INVESTIGATOR: Michael Garvey, M.D.

### PROJECT TITLE:

**Naturalistic versus Intensive Continuation Treatment of Mood Disorders** 

#### **SUMMARY:**

The major objective of this project was to compare the costs and therapeutic outcome of chronic mood disordered patients given intensive continuation treatment (ICT) versus naturalistic continuation treatment. We postulated that patients provided intensive outpatient treatment would have improvement in the quality of life and that their overall treatment costs would be less than the controls.

Sixty-five patients with chronic mood disorder were recruited and randomly assigned to receive usual and customary care or were provided intensive outpatient care with assessments at least every three weeks, educational sessions, counseling, and home visits if indicated. At the end of the two-year follow-up both groups had a detailed assessment of psychosocial functioning, episodes of illness, and costs of psychiatric and non-psychiatric care.

Psychiatric hospital costs for the two-year study were significantly reduced for the ICT group. There were more non-psychiatric costs associated with the ICT patients. Total health care costs were not significantly different between the two groups. Quality of life measurements were slightly better for the ICT patients, but these improvements were not statis tically significant.

Intensive continuation treatment programs are recommended for selected mood disordered patients. Use of non-psychiatric care needs to be monitored. Additional research should be performed to explore why intensive continuation treatment does not lead to higher quality of life ratings, and why it appears to be associated with greater non-psychiatric care costs.

MeSH terms: mood disorder; psychiatry; depression; compliance; treatment; health services research.



### PRINCIPAL INVESTIGATOR: Lois J. Geist, M.D.

### PROJECT TITLE:

Macrophage Function in the Pathogenesis of CMV Infection

### **SUMMARY:**

Objectives: Cytomegalovirus (CMV) pneumonia is a frequent cause of morbidity and mortality in immuno-compromised patients. While it is clear that CMV infection results in an inflammatory response in the lung, it is not known how CMV infection initiates pulmonary inflammation. One possibility is that the alveolar macrophage, an important immune cell in the lung, plays a pivotal role in determining the type of pulmonary response that develops after CMV infection.

Research Plan: These studies are designed to address the role of the alveolar macrophage in the development of the lung immune response to CMV. These studies look first at how lung immune cells respond to CMV infection. Then we will see what cytokines are produced in the response to CMV infection using animals that have genetic resistance or sensitivity to infection.

Methodology: We will perform these studies by assessing alveolar macrophage activation and cytokine production after CMV infection using a tissue culture model. We will then investigate these questions in an animal model to delineate the differences in cytokine production and cellular activation signals. This animal model takes advantage of genetic differences in different mouse strains. Some mouse strains are more likely to develop infection after CMV infection than other mice are. After we infect both strains of mice, we will look for differences

in cytokine production and other activation factors in the lung.

Findings: To date I have demonstrated that the alveolar macrophage may not play a major role in the response to a blood stream infection, but may have a bigger role in the response to an airborne infection. I have also demonstrated that mice that are sensitive to CMV infection produce different types of cytokines in the lung tissue after infection compared to mice that are not sensitive to CMV infection. I have also shown that certain signals are activated in the lungs of sensitive animals that are not seen in resistant animals. Using tissue culture, I have also been able to demonstrate that macrophage-like cell lines respond differently, depending on the animal source of the cell line. I have also shown how normal human alveolar macrophages respond to infection with CMV in a tissue culture model. These studies provide the first information on the mechanism whereby CMV infection induces the pulmonary inflammatory response. Further studies may help us to identify individuals that may be at higher risk for the development of CMV pneumonia after infection, particularly in the organ transplant population.

MeSH terms: inflammation; cytokines; signal transduction.



### PRINCIPAL INVESTIGATOR: Sarah L. George, M.D.

### PROJECT TITLE:

Virologic and Immunologic Analysis of Sero-silent Hepatitis C Infection

### **SUMMARY:**

Over 3 million Americans (1.5% of the population) are chronically infected with hepatitis C virus. Patients with chronic hepatitis C are at high risk of developing chronic liver disease, cirrhosis, and liver cancer. Currently available

treatments for hepatitis C such as interferon are only partially effective. Veterans are a recognized risk group for hepatitis C infection: an estimated 3% of veterans are infected, and 27% to 41% of veterans with alcoholic liver disease are infected with hepatitis C. Many veterans who required transfusions for service-related injuries were potentially exposed to hepatitis C-contaminated blood products prior to the advent of effective blood bank screening in 1990. The usual method of diagnosing hepatitis C infection is to test patient blood serum or blood plasma, although hepatitis C can also be found inside blood cells. Our laboratory developed a method to detect hepatitis C in whole blood samples, thus measuring the virus present in both serum and blood cells. Using this system, we have demonstrated that patients with chronic liver disease including cirrhosis frequently have detectable hepatitis C viral genetic material in their whole blood even though they tested negative for hepatitis C in serum or plasma. In addition, our test has demonstrated that hepatitis C may persist in whole blood during treatment with interferon when plasma tests indicate that the virus has been cleared. It appears that finding hepatitis C in whole blood may be predictive of relapse after completion of treatment. Our studies are designed to see if the reason hepatitis C infection is missed is due to changes in the genetic sequences of the virus, thus making the current test methods inaccurate, or whether the increased sensitivity of our test simply identifies people who have very low level infection. Thus far we have obtained partial viral sequences from 18 patients, and are working to extend our current sequence database to include more hepatitis C genes. We are also working to determine the effects of alcohol consumption on the growth of hepatitis C virus, since alcohol and hepatitis C appear to work synergistically to cause liver disease. We hope to broaden our understanding of how hepatitis C is able to survive in infected patients despite immune defenses, and how alcohol contributes to the liver damage caused by hepatitis C.

MeSH terms: virus; hepatitis C; liver disease; interferon; cirrhosis; plasma; serum; whole blood; alcoholic liver disease; immunity.

PRINCIPAL INVESTIGATOR: Nancy E. Goeken, Ph.D.

### PROJECT TITLE:

The Immunobiology of Bone Marrow Transplantation

### **SUMMARY:**

Bone marrow transplantation is a successful medical treatment for leukemia and some other potentially fatal blood diseases. In this treatment the patient requires high doses of chemotherapy and radiation to kill the leukemic cells. However, his/her bone marrow cells are also damaged by this treatment and must be replaced for the patient to survive. Ideally the marrow replacement comes from a perfectly matched brother or sister. Unfortunately only about 30% of such patients have an appropriately matched family members. Until a few years ago, patients without a suitable family donor were usually fated to die of their disease. Because of this, the Department of Veterans Affairs Medical Center and the University of Iowa, lowa City, began evaluating the potential usefulness of matched, but unrelated volunteer bone marrow donors. The marrow from such donors has, in fact, provided long term survival and even cures for some patients, although the overall incidence of complications has been higher than in those patients with family donors. The goal of this research proposal is to discover which genetic and immunological factors are mismatched between patients and unrelated donors and cause the serious, even fatal, complications of this type of bone marrow transplantation. This type of research has the potential to impact directly on clinical care. If the factor producing the complications of bone marrow transplantation were fully understood, this life saving treatment could be confidently offered to the thousands men, women, and children who are now destined to die of their leukemia.

MeSH terms: leukemia; bone marrow transplantation; transplantation; immunology; rheumatology.



PRINCIPAL INVESTIGATOR:

Thomas H. Haugen, M.D. Ph.D.

### PROJECT TITLE:

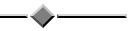
Regulation of Bovine Papillomavirus-1 Early Gene Expression

### **SUMMARY:**

Papilloma virus infection is considered a major cause in the development of cervical carcinoma in women. The factors that lead to cancers are complex. It appears that genital human papillomavirus (HPV) types as well as the model bovine papilloma virus (BPV-1) contain genes for proteins that contribute to cancers arising from cells they infect. We have shown that the expression of these genes in BPV-1 are regulated by complex interactions between multiple cellular proteins as well as other proteins provided by the infecting BPV-1 virus. Both the cellular and viral proteins exert this regulation by binding to specific regions of the viral DNA. We have shown that the viral DNA binding protein called E2 is a critical component of papillomavirus gene control. This protein can either elevate or decrease the expression of viral genes. On the other hand, in the early stages of infection only cell proteins are present. We have shown that efficient propagation of BPV-1 in tissue culture cells is dependent on these cellular factors. Once established in these cells however, the importance of cell proteins is reduced due to the presence of viral encoded regulatory proteins most likely including the E2. We conclude that the cellular factors are limiting in initiation of papillomavirus infection. Establishment of papillomaviral infection is a critical element in papillomaviral induced carcinogenesis. Further understanding of papillomaviral infection may

provide potential targets for therapeutic intervention.

MeSH terms: papillomavirus; neoplasms; gene expression; DNA; microbiology; pathology; oncogenes.



#### PRINCIPAL INVESTIGATOR:

William G. Haynes, M.B.Ch.B., M.D.

### PROJECT TITLE:

Hyperglycemia, Insulin Resistance and Endothelial Function in Human Diabetes

### **SUMMARY:**

Diabetes mellitus and its blood vessel complications account for 10-15% of admissions to VA hospitals. Diabetes mellitus substantially increases the risk of atherosclerotic cardiovascular disease (heart attack, stroke). Diabetes appears to damage the inner layer of cells that lines all blood vessels, (called the endothelium). Damage to the endothelium may then contribute to atherosclerosis through altered generation of hormones that control blood vessel function.

However, the mechanisms causing endothelial dysfunction in diabetes are unclear. Some investigators suggest that high insulin levels may cause vascular disease; others suggest a direct effect of high blood glucose. The <u>overall goal</u> of this project is to test the hypotheses that diabetes mellitus causes endothelial dysfunction through hyperglycemia; and that this occurs through generation of free radicals. The function of the endothelium is assessed in patients with type I and II diabetes and non-diabetic subjects using forearm blood flow measurements and ultrasound techniques. The following aims are being addressed:

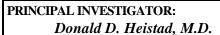
Aim 1 will examine whether acute perturbations in blood glucose concentrations alter endothelial function in human conduit or resistance vessels.

Aim 2 will investigate whether the endothelial dysfunction of diabetes can be corrected in the forearm vasculature by chronically reducing plasma glucose concentrations, and whether improving insulin sensitivity differentially alters endothelial function.

Aim 3 will explore whether reducing free radical induced oxidant stress by anti-oxidant therapy improves forearm and coronary endothelial function in diabetes.

We have now shown that the insulin sensitizing agent metformin (Glucophage) appears to improve blood vessel function in patients with type 2 diabetes. This improvement is substantially greater than that observed in patients receiving the standard sulfourea agent glipizide These results suggest that insulin resistance, rather than hyperglycemia, may be the most important cause of blood vessel damage in diabetes. These results may help in the development of more effective treatments to prevent the complications of diabetes mellitus.

MeSH terms: diabetes; nitric oxide; endothelium; oxidant stress; insulin resistance, human.



PROJECT TITLE:
SOD Gene Transfer and
Vascular Function

### **SUMMARY:**

Subarachnoid hemorrhage (SAH), or bleeding from blood vessels to the brain, forms a clot on the surface of the brain. SAH is frequently associated with spasm of brain blood vessels, which produces a stroke. The goal of this project is to use gene transfer of superoxide dismutase (SOD) to brain blood vessels, to study mechanisms that lead to spasm and perhaps ultimately to prevent vasospasm and stroke.

Gene therapy is at an early stage of development. Despite great obstacles this approach has considerable promise. Gene transfer to brain blood vessels is attractive because surgery for these vessels is difficult, and major clinical problems remain resistant to treatment. The opportunity to develop new methods to study cerebral vascular biology, with potential for unique therapeutic approaches, is extremely attractive.

Gene therapy to prevent vasospasm after SAH is potentially feasible. When a neurosurgeon places a clip on a bleeding vessel to the brain, it may be possible to administer a specially engineered virus that contains a gene to prevent vasospasm. We have constructed a special virus with a gene that produces SOD. After we give the virus, we have found that SOD is released into the cerebrospinal fluid that surrounds the brain. Our goal is to use SOD to reduce oxidant injury to brain blood vessels. This approach will allow us to study mechanisms that produce spasm of brain blood vessels after SAH. If gene transfer is effective, it may be useful as a unique therapy to prevent vasospasm and stroke.

MeSH terms: cardiology; stroke; gene therapy; vasospasm



PRINCIPAL INVESTIGATOR:

Joseph A. Hill, M.D., Ph.D.

PROJECT TITLE:

Voltage-dependent K<sup>+</sup> Channels in the Cardiovascular System: Kv1.5 a-Subunit

### **SUMMARY:**

The heart is an electrical organ. Normal heart function depends on a complex system of electrical circuitry within each heart cell (myocyte). Numerous forms of heart disease – including infarction (heart attack), valvular disease, hypertension, and many more -- disrupt the electrical functioning (electrophysiology) of heart cells leading to arrhythmias. Arrhythmias -states where the heart beats too rapidly, too slowly, or irregularly – are sometimes benign (e.g. atrial fibrillation) but often can be rapidly fatal (ventricular tachycardia or ventricular fibrillation). Our understanding of normal cardiac electrophysiology is incomplete, and our understanding of how these electrical circuits go awry in disease is yet more incomplete. In our lab, we use genetic models of heart disease to study the role of specific proteins (ion channels) in cardiac electrical activity. In the project funded through the VA, we are studying a strain of mice we have engineered to lack a specific cardiac ion channel (Kv1.5). By analyzing the effects of this genetic deletion on heart electrical function, we will begin to understand what this ion channel protein does in the heart. By imposing experimental conditions mimicking disease, we will begin to understand how this channel contributes to the electrophysiology of heart disease. Our long-term goals are to help design therapies (especially antiarrhythmic drugs) that will prevent and/or abort lethal cardiac arrhythmias.

MeSH terms: potassium channels; electrophysiology; anti-arrhythmia agents.



PRINCIPAL INVESTIGATOR:

Daniel H. Hug, B.S., Ph.D.

PROJECT TITLE:
Role of Urocanic Acid in
Immunosuppression by
Ultraviolet Radiation

### **SUMMARY:**

Most of us think that sunlight is good for us and in some respects it is. For example it can be used to treat some skin diseases, it makes vital vitamin D in the skin, it lifts moods, and it cures hyperbilrubinemia. However, men and women should avoid or protect themselves from sunlight, because it causes skin cancer, photoaging of skin (wrinkles), and suppresses the immune system. This suppression may impair our ability to resist infectious disease and possibly many forms of cancer. This laboratory has published many papers on urocanic acid (UCA) beginning in 1968.UCA is a substance in skin that forms a product, cis-UCA in sunlight that causes cellular immunity to be diminished. There is also some evidence that UCA is involved in photoaging. We have discovered that well-known bacteria (M.luteus) metabolize cis-UCA, and this organism is the first one shown to have this activity. We identified and characterized a new enzyme that is a key to these biochemical conversions. We also showed that certain commercial skin products kill this bacterium. M. luteus lives on human skin and by removing UCA, it may be beneficial to prevent skin aging and general loss of immunity. When individuals are protein malnourished, UCA levels increase dramatically, and we have proposed in two reviews that protein malnutrition makes persons very susceptible to a loss of immunity when they are exposed to ultraviolet(UV) light. The UV can come from the sun and also from fluorescent lights. People around the world are malnourished and protein malnutrition also occurs, for example, in a subgroup of individuals who suffer from alcoholism. Understanding the mechanism by which UCA "turns down" the immune system may lead to treatment or prevention of skin cancer, infectious disease, autoimmune disease, contact hypersensitivity and rejection of transplanted organs.

MeSH terms: immunity; photomedicine; skin neoplasms; urocanic acid; skin; protein malnutrition; bacteria



Gary W. Hunninghake, M.D.

#### PROJECT TITLE:

Silica or Asbestos Upregulation Tumor Necrosis Factor Gene

### **SUMMARY:**

Exposure to inhaled particles, like silica and asbestos, is a major health hazard. Many veterans have already had significant exposure to these agents. The health effects of exposure to silica and asbestos often are not detected for 15-20 years; thus, even though veterans may not currently be exposed, many who were exposed to these agents in the past will continue to develop lung disease. Currently, there is no therapy for these diseases. It is thought that silica and asbestos cause lung disease by causing inflammation in the lung. The purpose of these studies is to determine how these particles cause inflammation in the lung. We will obtain human lung macrophages from volunteers and expose their cells to silica or asbestos. We will then determine if exposure to the particles triggers release of compounds that are known to cause inflammation. If this is the case, we will then determine if silica or asbestos turn on the genes that produce the compounds that cause inflammation. We will also determine how this occurs. The ultimate goal of the studies is to develop new therapies to control the inflammation triggered by silica and asbestos. If this can be done, it will help many veterans who have been exposed to these particles and also result in a large savings in health care costs.

MeSH terms: pulmonology; respiratory diseases; pathology; silica; asbestos; macrophages; TNF; IL-1.

### PRINCIPAL INVESTIGATOR: Daniel Kahn, M.D.

PROJECT TITLE:

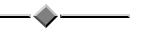
Phase I/II Study of Sm-153-Lexidronam for the Treatment of Patients with Refractory Rheumatoid Arthritis

### **SUMMARY:**

Rheumatoid arthritis in men and women can be a significant and disabling chronic disease. In those with rheumatoid arthritis there is a substantial portion of patients who will develop a severe, progressive and disabling form of the illness within five years of the on-set of their disease. A significant fraction of these patients are refractory to standard therapies, which includes Asprin, non-steroidal anti-inflammatory drugs (such as Motrin) and variety of diseasemodifying medications. It is in this group of patients in whom their arthritis remains active despite the full compliment of interventional therapies. We are investigating the use of a novel radioactive agent to alleviate pain and possibly change the course of the patient's rheumatoid arthritis. The purpose of the present study therefore is to evaluate the safety and the preliminary efficacy of varying doses of Samarium-153 administered as a single IV injection to patients with refractory rheumatoid arthritis. The patients are selected after having failed conventional therapy. These patients undergo a conventional bone scan to determine if the joints, which are causing problems, will take up sufficient amount of Samarium-153. If this is the case, patients will receive a single dose of therapy (it can be repeated in those that respond following three months). Follow-up of pain, function, and certain laboratory values are carried out over the following months. It is hoped that if this approach to treatment of this patient group is successful, a much larger, multi-centered study will be initiated to verify the results of the current

project (which is being performed only at the Iowa City VA Medical Center).

MeSH terms: rheumatoid arthritis; radioisotope therapy.



# PRINCIPAL INVESTIGATOR: Randy H. Kardon, M.D.

### PROJECT TITLE:

Objective Testing of the Visual Field Using Pupillary Contraction to Light: Comparison of Pupil and Visual Responses

#### **SUMMARY:**

An automated form of "pupil perimetry" has been devised to obtain objective information about the sensitivity of the field of vision in normal subjects and in patients with visual pathway damage. The timing and amplitude of pupillary contractions to focal light stimuli were recorded using a computerized infrared pupillometer linked to an automated perimeter. The computerized pupillometer records pupil movements to light stimuli at 60 times per second using an infrared video camera connected to a special computer board. The automated perimeter, also controlled by the same computer, projects small spots of light at different locations into an illuminated bowl which the patient views. When a spot of light is seen by the patient, a small pupil contraction is recorded by the computer, but if the light is not seen, the pupil doesn't contract, indicating damage. Pupil and visual sensitivity were compared at the same 76 locations across a 30 degree field of view. Pupil perimetry and standard visual threshold perimetry showed matching areas of defects in the visual fields of patients with optic nerve disease due to deprivation of blood flow or compression from tumors. However, the visual and pupillary responses matched only poorly in damage from glaucoma or optic neuritis (inflammation).

Patients with isolated strokes of the occipital cortex (visual processing area of the brain) showed matching visual and pupillary fields, suggesting an influence of higher functioning brain centers on the pupillary light reflex. Pupil perimetry is a useful, objective form of visual field testing and can be used to compare the effect of disease on the visual and pupillary pathways. Our preliminary studies have shown that pupil perimetry is more sensitive than subjective, visual responses for detecting early signs of damage to vision. It is easier, faster, and more objective than other forms of visual field testing, and thus should improve our ability to detect visual problems sooner, when they can still be treated.

MeSH terms: ophthalmology; vision; pupillary light reflex; neuro-ophthalmology; eye disease.



# PRINCIPAL INVESTIGATOR:

Lawrence P. Karniski, M.D.

# PROJECT TITLE:

Identification and Characterization of a Human Renal Sulfate Transporter

# **SUMMARY:**

Kidney stones afflict approximately 500,000 Americans each year, with the majority of these stones composed of the mineral calcium oxalate. Oxalate is a compound found in several food sources and it is also produced as a biochemical waste product in humans. Following its ingestion or production, oxalate is excreted in the urine; however, if the kidney excretes too much oxalate, kidney stones are formed. Investigators have discovered that the excretion of too much oxalate by people with kidney stones may be a genetic defect. We have recently identified a protein in the kidney that is involved in the transport of oxalate and sulfate from the blood to the urine. The gene encoding this protein was first identified in fibroblast cells and we have now isolated the DNA encoding this protein from the human kidney. Interestingly, a similar protein has been identified in the rat kidney. The objective of our current work is to purify the human oxalate transport protein, then analyze its structure and biochemical characteristics. Antibodies will also be made to the purified protein so that related proteins can be detected in other tissues. The location and function of the human and rat oxalate transport proteins will be compared to see if the rat kidney can be used as a model to gain an understanding of oxalate transport in the human kidney.

A second set of studies is examining the protein called Pendrin, which is defective in a congenital disorder called Pendred syndrome. Patients with this disorder develop hearing loss and have thyroid abnormalities. Pendrin normally functions to transport chloride and iodide in and out of cells. We are currently examining how defects in the pendrin protein can lead to deafness and thyroid disorders.

MeSH terms: nephrology; ion transport, kidney tubule; kidney calculi; oxalates; iodides; chlorides.



# PRINCIPAL INVESTIGATOR:

Louis V. Kirchhoff, M.D., M.P.H.

# PROJECT TITLE:

**Recombinant Assays For Diagnosing Chagas Disease** 

### **SUMMARY:**

Trypanosoma cruzi is the protozoan parasite that causes American trypanosomiasis, or Chagas disease. This illness is a major cause of morbidity and death in Latin America, and tens of thousands of *T. cruzi*-infected immigrants from Chagas-endemic countries now reside in the U.S. Identification of infected persons is important so that appropriate monitoring and treatment can be undertaken, and also to prevent transmission of the parasite by blood transfusion. Conventional

blood tests, which are manufactured with derivatives from parasites grown in the laboratory, often give unreliable results, causing misdiagnoses in clinical settings, transmission of the parasite by transfusion, and the needless discarding of false-positive donated units in blood banks. The goal of this project is to develop an improved blood test for detecting T. cruzi infection through the application of genetic engineering. The general approach involves production of parasite proteins in bacteria and using them as targets in test kits for detecting specific anti-T. cruzi antibodies. In preliminary work several blood tests based on parasite proteins produced in the laboratory were tested for their capacity to distinguish between T. cruzi-infected and uninfected persons. The current phase of this project involves determining the accuracy of the test that proved to be the best in the latter experiments by testing approximately 10,000 blood specimens obtained from geographically and medically diverse persons with and without *T. cruzi*-infection. The data produced in this second phase of the project will be used as the basis for a 510(k) application to the FDA for marketing clearance of the test kit. This innovative application of genetic engineering to the development of a test for detecting *T. cruzi* infection will provide the basis for more accurate clinical testing and more effective blood bank screening programs in Latin America countries and also in the U.S.

MeSH terms: Chagas disease; diagnosis; genetic engineering; blood test; antibody; *Trypanosoma cruzi*.

PRINCIPAL INVESTIGATOR: Ulla C. Kopp, Ph.D.

PROJECT TITLE:

Afferent Renal Nerves: Role of Neurotransmitters

#### **SUMMARY:**

Our research is focused on the communication between our two kidneys. Obstruction of one ureter by a kidney stone, blood clot in the ureter, spasm (constriction) of the ureter due to inflammation of the ureteral wall would obstruct the outflow of urine from that kidney, ultimately leading to increased retention of water and salt. Ureteral obstruction is very common in men, especially in men over 60. The increased water and salt retention would increase body fluid and result in increased blood pressure. However, we have identified a mechanism that may minimize the excessive water retention during obstruction of one kidney. During ureteral obstruction, pressure within the obstructed kidney increases due to continued urine production. The increased pressure triggers sensors within the kidney that send a signal to the brain to increase urine production from the opposite non-obstructed kidney. Thus total urine output from the two kidneys may be unaltered. This compensatory response from the other kidney (i.e. increased urine production) is reduced in disease states such as hypertension. We have found that prostaglandins and the neurotransmitter substance P are involved in the triggering mechanisms of the sensors activated by the increased pressure in the kidney during ureteral constriction. Constriction of the ureter increases the release of prostaglandins which in turn increases the release of substance P. Substance P triggers the activation of the nerve signal from the kidney to the brain. Interestingly, pain due to kidney stones is treated with inhibitors of prostaglandins, eg., ibuprofen. It is well known that the sensation of pain is triggered by substance P. Our findings showing that prostaglandins elicit the release of substance P suggest that the pain relief with ibuprofen is related to decreased release of substance P. We have also shown that the lack of a compensatory response from the other kidney during

constriction of one ureter in hypertensive rats is related to reduced release of substance P and reduced activation of receptors triggered by substance P. Clarifying the mechanisms involved in the responses to ureteral constriction in normal and hypertensive rats may lead to better treatment of ureteral obstruction and hypertension.

MeSH terms: nephrology; cardiovascular disorders; hypertension; ureteral obstruction; sensory receptors; renal pelvis; prostaglandins; substance P.



PRINCIPAL INVESTIGATOR:

Arthur M. Krieg, M.D.

PROJECT TITLE:

B cell Activation by Oligodeoxynucleotides with Cpg Motifs

# **SUMMARY:**

It has long been recognized that DNA is the genetic material for all living organisms. However, we have recently discovered a structural feature of DNA that can also function as a signal to alert white blood cells to the presence of infection. Several types of white blood cells are able to detect a simple yet characteristic pattern in DNA molecules from bacteria and viruses that distinguishes these DNA molecules as being foreign because it is not present in our DNA (or in the DNA of other vertebrates). In response to this DNA signal, which we call a "CpG motif," white blood cells rapidly become activated and start secreting chemical messengers called cytokines which rally a broad range of immune defense mechanisms that help to fight off the infection. A type of white blood cell called the B cell also secretes antibodies that attack invading germs. The purpose of our project is to determine how this CpG motif works to activate B cells.

Our preliminary studies indicate that B cells take up DNA around them and transport it into the cell nucleus. If the DNA is from other "friendly" cells, then it will not contain a CpG motif and will not activate the cell. However, if any germ DNA is around, then proteins in the nucleus of the B cell are able to bind to the CpG motifs in that DNA, and these proteins turn on certain genes that will activate the B cell and help to begin the immune reaction. Our project seeks to identify the protein or proteins that recognize the CpG motif and to determine how these proteins turn on the other genes that are needed for immune defence.

MeSH terms: immunology; transcription; cytokines; B cells; lymphocyte activation.



PRINCIPAL INVESTIGATOR:

David J. Kusner, M.D.

PROJECT TITLE:

The Regulation of Phospholipase D In Phagocytic Leukocytes

# **SUMMARY:**

Monocytes, macrophages, and neutrophils (phagocytic leukocytes) are critical components of the inflammatory response. These cells constitute the cellular basis of innate immunity, and in concert with lymphocytes, promote the development of specific immune responses. However, the activation of phagocytic leukocytes has both beneficial and detrimental effects. These cells ingest and kill invading microorganisms, but their activation also results in damage to host tissues at inflammatory sites. This latter capacity for damage to human organs and tissues is manifest in such medically important diseases as sepsis, the acute respiratory distress syndrome (ARDS), heart attacks, and strokes. Our ability to modulate phagocyte responses, in order to maximize immune defenses and minimize injury to humans, is limited by our lack of understanding of the mechanisms which regulate the activation of phagocytic leukocytes.

Phospholipase D (PLD) is an enzyme that is rapidly activated during the early stages of the stimulation of leukocytes. PLD has also been linked to the major antimicrobial and tissuedamaging functions of these cells, including generation of reactive oxygen intermediates, secretion of microbicidal and inflammatory compounds, and phagocytosis (or ingestion) of microorganisms. However, the regulations of PLD, and subsequently, its activation of leukocyte responses, are poorly understood. The aim of this project is to characterize the biochemical mechanisms that regulate PLD in monocytes, macrophages, and neutrophils. Understanding the means by which this essential enzyme controls leukocyte responses may provide opportunities to modulate the state of activation of these cells, to promote host defense and avoid the deleterious consequences of excessive leukocyte activation.

MeSH terms: infectious diseases; phospholipase D; macrophages; leukocytes; monocytes; neutrophils; signal transduction.



PRINCIPAL INVESTIGATOR:

Kathryn Lamping, Ph.D.

PROJECT TITLE:

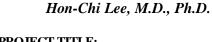
Responses of the Coronary Circulation in Enos-deficient Mice

#### **SUMMARY:**

Epidemiological studies have demonstrated a difference in the incidence of cardiovascular disease in men and women. The incidence of cardiovascular disease is approximately half in premenopausal women compared to age matched men until the age of menopause when cardiovascular disease increases in women much more rapidly until it is similar to men. These

gender differences in cardiovascular disease in men and women have been attributed to a protective effect of estrogen on lipid profiles and or vascular reactivity. Recent studies have suggested that the ability of estrogen to increase nitric oxide synthase the enzyme responsible for the production of nitric oxide is the primary mechanism involved in the protective effect. Three different forms of the enzyme nitric oxide synthase (NOS) have been identified: endothelial NOS, neuronal NOS and inducible NOS. We have recently examined vascular responses of arteries obtained from male and female mice deficient in the gene expressing endothelial NOS to determine the contribution of endothelial NOS in the protective effects of estrogen. In isolated carotid arteries from normal mice contractions to serotonin were greater in males compared to females. Maximal contractions to the thromboxane analog U46619 were similar. Deletion of a single and both copies of the gene for endothelial NOS increased contractions to serotonin in arteries from both male and female mice but the effect was greater in arteries from female mice. Maximal contractions to U46619 were not altered by deletion of endothelial NOS. These findings suggest that gender difference in responses to serotonin are predominately mediated by differences in endothelial NOS. We are examining whether similar responses are present in the coronary circulation since the regulation of vascular tone appears to differ based on the source of vessels. These studies will help to determine mechanisms involved in the gender differences in cardiovascular disease and may provide important information concerning potential therapeutic approaches to preventing disease.

MeSH terms: cardiovascular system: endothelium, vascular; sex hormones; estrogens.



PRINCIPAL INVESTIGATOR:

# PROJECT TITLE:

**Mechanism of Alpha-2 Adrenergic Modulation of** Cardiac Electrophysiology

#### SUMMARY:

Approximately 80% of the 350,000 deaths each year from sudden cardiac arrests in this country are due to abnormal heartbeats. Adrenaline hormones are known to exert many different effects on the heart including regulation of the heartbeat and is thought to play an important role in the development of life-threatening abnormal heart beats. The different effects of adrenaline in heart cells are brought about through different receptors which are protein molecules on the surface of the cell that act as sensors for adrenaline, recognizing the hormone and executing different cellular functions. Hence, understanding how these receptors execute the adrenaline effects on the electrical impulse of the heart is crucial to our progress in the diagnosis and treatment of heart diseases.

Recently, we made a novel discovery that the specialized electrical conduction system in the heart has a type of receptor that has not been previously identified in the heart. Stimulation of these receptors by adrenaline has stabilizing effects on the electrical impulse of the heart and may protect the heart against the development of some types of abnormal heartbeats.

The proposed research is designed to study the mechanisms of action through which this new type of receptor affects the electrical properties of the heart. Using state-of-the-art technology, we will study the traffic of ions and electrical currents in single heart cells. We will determine the effect of adrenaline on the traffic of ion currents that may lead to changes in the electrical impulse of the heart. We will also determine the cellular factors that are essential for execution of the adrenaline effects through this receptor. The

results of our research may help us to know how electrical impulses and abnormal heartbeats are controlled by adrenaline. It may also help to improve our approaches in the diagnosis and treatment of abnormal heart beats.

MeSH terms: electrophysiology; pharmacology; ion channel; signal transduction.



PRINCIPAL INVESTIGATOR:

Steven R. Lentz, M.D., Ph.D.

# PROJECT TITLE:

Vascular Dysfunction in Hyperhomocysteinemia and Atherosclerosis

# **SUMMARY:**

Homocysteine is an amino acid that is found normally in blood. It is a by-product of methionine, which comes from protein in the diet. Like high cholesterol, high levels of homocysteine are common in the United States population. Several recent studies have demonstrated that elevated homocysteine in the blood is a risk factor for cardiovascular disease and stroke. Fortunately, homocysteine levels can be lowered by a vitamin called folic acid, which can be obtained from fruits and green vegetables in the diet, or taken as a vitamin supplement. It is not vet known, however, whether supplementation of the diet with folic acid will be sufficient to prevent heart attacks or strokes in patients with cardiovascular disease.

Our studies have shown that even slightly elevated levels of homocysteine can damage the lining of blood vessels. We found that blood vessels failed to relax normally when homocysteine levels were elevated, and that there was a tendency to form blood clots. Some of these abnormalities are similar to those seen with high cholesterol. In our current studies, we are using dietary and genetic models to

characterize mechanisms by which homocysteine damages blood vessels, and to define the role of folic acid deficiency in this process. These questions have important clinical consequences, since dietary intervention to lower plasma homocyst(e)ine has been proposed as a global strategy to decrease the prevalence of cardiovascular disease in the United States.

MeSH terms: atherosclerosis; blood coagulation; endothelium; folic acid; homocysteine; thrombin; thrombomodulin; thrombosis.



PRINCIPAL INVESTIGATOR: Ramon Lim, M.D., Ph.D.

PROJECT TITLE:

GMF as a Signaling Molecule

# **SUMMARY:**

The nervous system is the most complex system within the body. From the brain all the way down to the spinal cord, myriads of nerve cells form intricate connections resulting in the most complicated communication network found in nature. This organizational wonder not only makes possible the higher intellectual functions which are unique to human beings, but also is responsible for the basic activities necessary for daily life. Too often in the Neurology Clinic, we see veterans with the nervous system impaired in one way or another. These ailments include Alzheimer's dementia where the brain no longer is able to retain memory and execute judgment; Parkinson's disease where the brain is no longer capable of coordinating movements; brain tumors where abnormal cells invade and destroy the network of neural tissue; and spinal cord injuries where even simple daily activities such as walking and urination are rendered impossible. Over the years, our laboratory has engaged in the study of protein messages that nerve cells used to communicate with each other. These proteins,

called "growth factors", are extremely important in maintaining the proper structure and function of the entire nervous system. More recently we have extended our study to identify those messages that relay information from the surface of the brain cells to the command center (the nucleus) inside the cell. These intracellular messages, called "signal transducers" are responsible for the proper functioning of the cell including maturation, proliferation and regeneration, and are essential to the understanding of the disease mechanisms in brain tumors, Alzheimer's dementia, Parkinson's disease, spinal cord injury, and other neurological problems afflicting veterans. We are hopeful that our research into these mechanisms will improve the quality of life of many of our veterans.

MeSH terms: neurology; biochemistry; Alzheimer's dementia; Parkinson's disease; growth; brain injuries; nerve regeneration; neurons.



# PRINCIPAL INVESTIGATOR: David M. Lubaroff, Ph.D.

# PROJECT TITLE:

Inhibition of CD40-CD40L Interactions Augments Vaccine Therapy of Prostate Cancer

# **SUMMARY:**

Prostate cancer is the second leading cause of cancer death among males in the United States. The only systemic treatment for this cancer is the manipulation of androgens, a treatment that will result in growth inhibition of cells that are androgen-dependent, but has no effect on the population of malignant cells that are androgen-independent. These androgen-independent prostate tumor cells continue to grow and metastasize. Anti-tumor therapies for cancer,

including prostate cancer, have been limited, prompting many scientists to investigate the use of immunotherapies and gene therapies. Most of the immunotherapy studies for prostate cancer have concentrated on active non-specific therapy and adoptive or passive therapy, with only recent attention paid to the induction of antigen-specific immune responses. It is our contention that active immunization against antigens associated with prostate cancer will be more effective than active non-specific or adoptive/passive immunotherapy. Therefore, we have been pursuing a vaccination strategy based on vaccinia and adenoviruses that carry the gene for prostate specific antigen (PSA). Viral vectors have been used successfully in both gene transfer and vaccine therapy studies. Replication competent and replication deficient viruses expressing foreign proteins have been used to elicit immune responses to a variety of tumor antigens. We have been able to demonstrate that immunizations with both the attenuated vaccinia virus (NYVAC) and adenovirus (Ad), carrying the human PSA gene, can induce vigorous anti-PSA T-cell responses. Many gene and vaccine therapy protocols may necessitate repeated administration of the microbial vectors. The efficacy of two, three, or more injections of Ad has been shown to induce the production of antiadenoviral antibodies that limit the efficacy of the therapy. Previous studies have demonstrated that the administration of an antibody to the CD40 ligand (CD40L) will inhibit the production of anti-adenoviral antibodies and potentiate the transfer of genes to host cells. In our research we propose to treat prostate cancer, using a mouse model of the human disease, through the induction of anti-PSA T cells, augmented by the use of anti-CD40L antibody.

MeSH terms: immunotherapy; vaccines; prostate cancer.



# PRINCIPAL INVESTIGATOR: David M. Lubaroff, Ph.D.

#### PROJECT TITLE:

VA Research Enhancement Award Program (REAP): Cellular Activation in Prostate Cancer

#### **SUMMARY:**

This REAP award focuses on prostate cancer and will benefit the VAMC in many ways. Prostate cancer is the second leading cause of death among males in the United States. A very large percentage of the VAMC patient population is male and in the age bracket (50 and older) in which the incidence of prostate cancer is on the rise. The only systemic treatment for this cancer is the manipulation of androgens, a treatment that will result in growth inhibition of cells that are androgen-dependent, but has no effect on the population of malignant cells that are androgenindependent. These androgen-independent prostate tumor cells continue to grow and metastasize. The research in this REAP application has concentrated on cellular activation processes that impact prostate cancer. This includes the consequences of signaling through the CD40 molecule and its effects on tumor growth in vivo. Another cellular activation project will investigate signaling through the dopamine receptor and its role in the growth of androgen-independent prostate cancer cells. This androgen-independent population of cells grows progressively, even in the absence of androgens, ultimately causing the death of the patient. Understanding the mechanisms through which prostate cancer cells are stimulated to grow or to die will at the end translate into better therapy for the disease. The final project will investigate the immunologic mechanisms that occur following vaccine immunotherapy by the generation of antigen-specific immune response to antigens associated with prostate cancer cells. We all strive for a better and more powerful

method of treatment for this cancer and the extraordinary adjuvant properties of CpG ODN has been shown to induce a more vigorous immune response resulting in protection from tumor challenge. All the current projects will provide a better understanding of the biology of, and therapy for, prostate cancer. The collaborative, educational and training aspects of this REAP are both extremely important and will insure the continued development of a strong prostate cancer research center at the ICVAMC and the adjacent University of Iowa.

MeSH terms: activation; therapy; androgens.



Donald Macfarlane, M.D.

PROJECT TITLE:

Genetic expression changes in B-cells with Exposure to Bacterial DNA

# **SUMMARY:**

It is known that bacterial DNA has various effects on immune cells, some of them being: increased production of cytokines (chemical messages), increase in cellular proliferation, and lack of apoptosis (programmed cell death). All of these changes are regulated by genes within the cell's nucleus. We have cloned B-cells that are sensitive to bacterial DNA, and B-cells which are resistant to bacterial DNA. These resistant cells take in the bacterial DNA like the sensitive cells, however, they do not make cytokines, proliferate, and will undergo programmed cell death. We plan to study the genetic differences in these cells by various methods. One technique we will explore is called a differential display. In this technique, cellular genetic material is electrophoresed through a gel, and by looking at the banding patterns created by the genetic material, differences in expression can be found. These

bands are then excised and sequenced to determine which gene they represent. This is a very powerful technique that will allow analysis of many conditions and cells simultaneously. Another technique we have already been working with is transfection. Genetic material from cells that respond to bacterial DNA is placed in a vector and introduced into the cells which do not respond to bacterial DNA. The vector allow the genetic material to be expressed in the cell. The cells are then challenged with bacterial DNA, and the cells that respond are cloned. The introduced genetic material is then isolated and sequenced, again in an attempt to determine which genes are critical in this response. The goal of these experiments is to achieve a greater understanding of autoimmune disease, which may lead to ways to prevent it or treat it more effectively.

MeSH terms: bacterial DNA; autoimmunity; DNA; RNA.



PRINCIPAL INVESTIGATOR:

Donald Macfarlane, M.D.

PROJECT TITLE:

**Inhibition of Bacterial DNA-induced Immune Responses** 

#### **SUMMARY:**

Bacterial DNA has been shown to have effects on the mammalian immune system, both positive and negative. Positively, it can be added to vaccines to make them more effective.

Negatively, it has been associated with rheumatoid arthritis and lupus. Our research focus has been on inhibiting the negative effects of bacterial DNA, in an effort to have more effective treatments with fewer side effects than are currently available for these diseases. We have tested over 500 different compounds to date, and we are attempting to ascertain a structure chemical relationship to help us design compound which work at low concentrations and

have minimal negative effects. The compounds are tested and evaluated for their ability to inhibit cell-signaling events. In a collaborative effort with another investigator, some of these compounds have been tested in mice to see how they work in an organism instead of in a culture dish. We plan to use a mouse model to test certain compounds for efficacy and toxicity. Our goal in this project is to create more powerful drugs for use in treating autoimmune diseases, and perhaps determine how these drugs work to inhibit the cellular response to bacterial DNA.

MeSH terms: immunity; bacterial DNA; antimalarials.



PRINCIPAL INVESTIGATOR:

Rama K. Mallampalli, M.D.

PROJECT TITLE

Lipid Regulation of

Cytidylyltransferase in Adult
Lung

#### **SUMMARY:**

The infant respiratory distress syndrome (RDS) still remains a leading cause of morbidity and mortality in the preterm low-birth-weight infant. Chronic obstructive pulmonary disease, asthma, acute lung injury, and pulmonary fibrosis are common lung disorders in the US population which are also associated with high mortality and morbidity. All of these conditions are associated with deficiency of a material that provides an inner, protective lining to the lung, surfactant. Surfactant stabilizes the lungs and prevents their collapse. Recent studies have also shown that a toxic protein, termed tumor necrosis factor (TNF), participates in many of the lung disorders listed above. We hypothesize that TNF decreases surfactant production, thereby contributing to the process of tissue inflammation and injury

observed in lung disease. Our research investigates how TNF decreases production of surfactant synthesis in the lung. Our research proposal specifically focuses on how TNF controls a key surfactant production enzyme, cytidylyltransferase (CT). The specific hypothesis underlying these studies is that cytidylyltransferase (CT) activity in the lung is inhibited by TNF, thus leading to altered surfactant synthesis. Thus, an understanding of how this protein controls surfactant metabolism at the biochemical level might be critical before devising newer therapies with agents that might oppose the actions of TNF.

MeSH terms: surfactant; cytidylyltransferase; tumor necrosis factor.



PRINCIPAL INVESTIGATOR:

James B. Martins, M.D

PROJECT TITLE
Purkinje Origin of Ventricular
Tachycardia

### **SUMMARY:**

Sudden death accounts for over 300,000 deaths due to heart disease every year in this country. The overwhelming majority of these patients have rapid heart rhythm disturbances, ventricular tachycardia (VT), associated with blockages in the coronary arteries that are associated with old scars or new limitations of blood supply to the bottom chambers of the heart. Up to two-thirds of those patients with VT and old scars may be cured by removing the lining of the scar that is the site of specialized conducting fibers called Purkinje cells. The purpose of the present investigation is to ask the question whether these fibers are responsible for VT when a coronary artery is ligated in the dog or pig. This investigation found several lines of evidence to

suggest that Purkinje fibers give rise to VT occurring spontaneously or induced electrically in the first hours after coronary artery occlusion. First we found that 3-D electrical mapping revealed that 50% of VTs originate from Purkinje tissues in the ischemic zone. Second, we found that VT arises from focal sites and are uniquely responsive to drugs that stimulate alpha-2 adrenergic receptors found only on Purkinje tissue. Clonidine (used clinically) and UK14304, prevent VT when it originates in Purkinje tissue in this model. Third, tissues removed from the focus of VT in dogs and pigs reveal abnormalities suggestive of a specific mechanism of arrhythmia limited to Purkinje tissues. This mechanism occurs in Purkinje tissue more frequently than in muscle from the lining of the ventricle and is more frequently is blocked by clonidine or UK14304. These studies in animals suggest the possibility that Purkinje tissue and the unique responsiveness to alpha-2 adrenergic receptor agonists may be useful targets for prevention of sudden death in human beings.

MeSH terms: electrophysiology; action potentials; heart myocardium; ischemia; arrhythmias.



PRINCIPAL INVESTIGATOR: Nina A. Mayr, M.D.

PROJECT TITLE

Radiation Therapy Oncology
Group (RTOG)

#### **SUMMARY:**

The Radiation Therapy Oncology Group (RTOG) is a national cooperative study group conducting clinical cancer research primarily in the area of radiation oncology. In addition, the RTOG has a strong interest in multimodality treatments and in devising new treatment strategies in the treatment of cancer. Since its foundation in 1968, 245 clinical trials have been conducted by the

RTOG. Presently, the RTOG has 43 active clinical protocols for various cancer sites, including the head and neck area, central nervous system, lung cancer, lymphoma, gastrointestinal, male urinary tract, gynecological cancers, and metastatic tumors. New protocols are constantly being developed. To date, approximately 52, 000 patients have been entered on clinical trials since the founding of RTOG. There are presently over 200 member institutions throughout the nation, and 40% of radiation oncologists in this country are RTOG members. The primary goals of the RTOG are to increase survival in various types by improving local-regional tumor control by means of radiation therapy, and where appropriate, by investigating the role of systemic therapy; to evaluate new treatment modalities; to improve cancer treatment outcome; and decrease morbidity by sparing structure and normal organ function and by increasing control and/or survival by the use of radiation therapy alone or in careful integration with chemotherapy and resection. The RTOG is also involved in the search for new means of preventing second or subsequent malignant tumors in patients cured of their malignancy. The University of Iowa/VAMC became a member of the RTOG in January 1995. Presently, 8 protocols are open for patient entry, and several will be available in the near future.

MeSH terms: radiation therapy; chemotherapy; carcinoma; clinical trials; multimodality therapy.



PRINCIPAL INVESTIGATOR:

Michael L. McCormick, Ph.D.

PROJECT TITLE

**Eosinophil-mediated Formation** of Reactive Tyrosine Species

# **SUMMARY:**

Eosinophils are an important component of the immune response to various chronic parasitic

infections. When stimulated, eosinophils generate reactive oxygen species (ROS) and release the contents of their granules, including the specific granule protein eosinophil peroxidase (EPO). In asthma, however, eosinophils are overrecruited to the airways, and it has been suggested that activation of these cells in the asthmatic lung may play a role in the observed tissue damage. Previous work in our laboratory has shown that other peroxidases similar to EPO can lead to the formation of various reactive compounds capable of altering the tertiary structure of proteins and leading to loss of function and/or enzyme inactivation. Our preliminary studies with EPO have shown that this enzyme also has these properties. We have hypothesized that these EPO-mediated reactions could be important not only in pathogen killing, but also in the normal tissue damage seen during eosinophil over-recruitment (e.g. asthma). We will examine this process in a cell-free system containing purified EPO, in cellular systems containing isolated eosinophils (from both peripheral blood and from tissue resident sites), and in intact parasite-induced mouse liver granulomas (containing 50% tissue-resident eosinophils). These studies will help define potential mechanisms involved in eosinophilmediated host defense against parasitic infection as well as eosinophil-mediated inflammatory tissue injury in situations such as asthma and other allergic reactions.

MeSH terms: eosinophils; parasitic diseases; asthma; reactive oxygen species; granuloma; peroxidase.



PRINCIPAL INVESTIGATOR: Stephen E. McGowan, M.D.

PROJECT TITLE:

**Influences of the Extracellular Matrix on Elastin Production** 

SUMMARY:	

My research is directed at understanding how the cells and proteins that form the scaffolding of the alveolar region of the lung grow and repair themselves. When such agents as cigarette smoke, toxic dusts and chemicals, or harmful cells damage the lung, it can repair itself under some but not all circumstances. In most cases the repair is not complete, so that over time, there is a slow loss of lung function. One way to prevent the loss of lung function is to eliminate the harmful agents, but this approach is not always successful. Therefore it is important to develop ways to reverse damage that has already occurred. Unfortunately the currently available treatments for emphysema and lung fibrosis cannot reverse the damage that has taken place, and in many cases cannot even successfully retard the damage that is in progress. This leads to frequent and costly hospital admissions during which drugs are administered to help the veteran through an immediate crisis, only to have him return in a short time with the same or even greater problems. My research goal is to develop our understanding of the biochemistry of the lung's structural framework by analyzing how lung cells initiate and regulate the repair process. This involves studying the regulation of the production of the major elastic protein in the lung, elastin, which is only produced during lung growth in childhood or after damaging lung diseases. We are learning how cells recognize that damage has occurred, how they grow to replace damaged cells, how they know to stop growing and produce extracellular proteins, and whether regulated cell death is involved in the repair process. We are particularly interested in how vitamin A and its derivatives increase elastin production. We are also studying how cells signal one another through extracellular proteins that are secreted into their environment that alter protein synthesis in surrounding cells. We are studying how these extracellular signaling proteins increase intracellular protein modification and gene expression. Armed with a better understanding of these basic processes, I am hopeful that we can develop drugs and other strategies that will offer a new hope in treating emphysema and lung fibrosis.

MeSH terms: emphysema; elastin; tropoelastin; retinoids.

# PRINCIPAL INVESTIGATOR: William M. Nauseef, M.D.

PROJECT TITLE:

Neutrophil Myeloperoxidase: Structure, Function, and Biosynthesis

#### **SUMMARY:**

The human immune system defends against invading microorganisms in multiple ways. The most immediate response to infection is recruitment of activated polymorphonuclear leukocytes (PMNs), the cells which make up "pus". The importance of normally functioning PMNs for optimal health is demonstrated by the frequent and severe infections seen in patients who lack PMNs, either from diseases such as leukemia, bone marrow suppression from chemotherapy, or inherited defects in PMN function. The most efficient system used by activated PMNs depends on two major components: toxic oxygen-derived products such as hydrogen peroxide, produced when PMNs are stimulated, and myeloperoxidase (MPO), a PMN granule protein secreted by activated PMNs. In the presence of hydrogen peroxide, MPO catalyzes the generation of hypochlorous acid (bleach) which the PMN uses to kill bacteria. The inherited absence of MPO is a common defect in the general population, occurring with a frequency of 1 in every 2,000 to 4,000 people. Our studies have suggested the defect reflects faulty synthesis and packaging of the precursor of MPO in the cell, resulting in a failure of the affected cells to succeed in producing normally functioning MPO. As a result, the affected PMNs kill bacteria less efficiently than do normal PMNs and fail to kill some fungi at all, a very significant problem for

patients who also have diabetes mellitus. Our current studies are directed at identifying the genetic basis for MPO deficiency and defining the biochemical consequences of the genetic mutation. The potential impact of our studies on the quality of clinical care is far-reaching, since the coexistence of diabetes mellitus and MPO deficiency makes the patient at heightened risk for acquiring clinically significant fungal infections. Identification of these patients will allow for more careful monitoring for signs and symptoms referable to fungal infection. Furthermore, genetic manipulation to correct the defect resulting in MPO deficiency would allow definitive elimination of the risks imposed by this genetic disorder.

MeSH terms: infectious diseases; biochemistry; neutrophils; granulocytes; myeloperoxidase; host defense; granule proteins; lysosomes; lysosomal proteins; biosynthesis; diabetes; fungal infection.



PRINCIPAL INVESTIGATOR:
William M. Nauseef, M.D

#### PROJECT TITLE:

P57, a Coronin Homologue and Actin-binding Protein Important for Assembly of the Nadph Oxidase of Human Neutrophils

### **SUMMARY:**

Chronic granulomatous disease (CGD) is an inherited disorder characterized by recurrent infections due to defects in an enzyme called NADPH oxidase. This enzyme assembles on the surface membrane of neutrophils and generates reactive oxygen species (such as superoxide ions) to kill invading bacteria. The oxidase is assembled from six proteins subunits: gp91phox and p22phox, rac, p40phox, p47phox and p67phox. CGD can be caused by mutations in the

"phox" proteins. We are interested in exploring the role of the actin cyotskeleton and actin binding proteins in assembly of the NADPH oxidase. Actin is an important structural protein in all cells which polymerizes into filaments. The spatial arrangement of these filaments gives cells their shape, and the filaments also act as "tracks" to localize proteins to specific intracellular locations in conjunction with other actin-binding proteins. We have recently developed an assay to identify new actin-binding proteins associated with the surface (plasma) membrane of human neutrophils. One of these proteins is called p57, and it is similar to a slime mold protein called coronin. Using the techniques of biochemistry and immunofluorescence microscopy we have shown that p57 is recruited to specialized areas of the neutrophil plasma membrane when these cells are ingesting invading bacteria. These areas are called phagosomes, and are also sites for assembly of the NADPH oxidase. We are currently investigating the hypothesis that p57 recruits p47phox, p67phox and p40phox to the phagosome to facilitate assembly of the NADPH oxidase, and we have evidence that the time course of p47phox association with phagosomes is altered in patients with X-linked CGD. A better understanding of neutrophil actin binding proteins such as p57 will further our understanding of phagocytosis in general, and assembly of NADPH oxidase in particular. It is our hope that these data will eventully lead to better therapies for CGD patients.

MeSH terms: chronic granulomatous disease; neutrophils; NADPH oxidase; actin; coronin; phagocytosis.



Gary E. Rosenthal, M.D.

### PROJECT TITLE:

Impact of Outsourcing VA Cardiac Surgery on the Cost and Quality of Care

#### **SUMMARY:**

Coronary artery bypass surgery (CABG) is a potentially effective treatment for many patients with ischemic heart disease. However, the maintenance of cardiac surgery programs requires substantial investments and may draw resources away from other programs, such as primary care. Moreover, many of the VA cardiac surgery programs perform relatively low volumes, which may be associated with poorer patient outcomes. Thus, outsourcing CABG and other cardiac surgeries to private sector hospitals may be cost-effective and allow the VA to focus service delivery in other high priority areas. However, little empirical data about the relative quality of CABG in VA and private sector hospitals is available to guide VA managers and policy makers. The primary objective of this research project is to define the potential impact of outsourcing cardiac surgery by comparing severity-adjusted mortality and patient perceptions of hospital quality in patients undergoing CABG in VA and private sector hospitals.

The study has two phases. In Phase I, mortality and length of stay in 43 VA hospitals that performed CABG in 1993-97 will be compared to mortality and length of stay in private sector hospitals in New York State and Northeast Ohio. Analyses will involve the development and validation of models to adjust for differences in patient risk factors across the three data bases. Fifteen patient risk variables that are defined and abstracted in nearly identical methods in the VA, New York, and Northeast Ohio databases have been identified for use in model development. Preliminary analyses reveal differences in patient risk factors across the three databases that may impact mortality rate comparisons.

In Phase II, patient satisfaction in randomly selected patients undergoing CABG in VA hospitals in 1998-2000 will be compared to patient satisfaction of CABG patients in a national representative normative database of private sector hospitals. Comparisons will be made using a previously validated and widely used method for measuring patient satisfaction that measures several distinct dimensions of hospital care. Analyses will adjust for differences in age,

health status, and other demographic and clinical factors between VA and private sector patients.

MeSH terms: cardiac surgical procedures; coronary artery bypass; quality of health care; outcome assessment; risk assessment; risk factors; hospital mortality; patient satisfaction; hospitals (veterans);



# PRINCIPAL INVESTIGATOR:

James Rossen, M.D.; Charles McKay, M.D.; Pamela Nerheim, M.D.; Michele Costigan, RN; Kathy Schneider, RN; Kathy Lilli, RN

# PROJECT TITLE:

Clinical Outcomes Using Revascularization and Aggressive Drug Evaluation (The COURAGE Trial)

#### **SUMMARY:**

The purpose of the research is to compare two treatment therapies in patients with coronary artery disease to determine which therapy is better. There are currently three kinds of approaches: treatment with medicines, coronary artery bypass surgery, and percutaneous transluminal coronary angioplasty (PTCA). This study will look at treatment with medicines compared to PTCA plus medications. PTCA has been used safely in patients for over 15 years. It is a technique for widening the narrowed arteries in the heart by either inflating a small balloon placed in the narrowed area or using a special device (rotoblator) to burrow through, or shave plagues of calcium from the narrowed areas. Sometimes a small expandable stainless steel mesh tube (stent) is placed in the artery to assist in keeping it open. It has not been proven whether those who have PTCA live longer, have fewer heart attacks, or have a better quality of life than those who only have medicine. The American Heart Association has provided guidelines for intensive treatment of patients with coronary artery disease with medicines. These medicines include aspirin, beta-blockers (drugs that decrease the work of the heart), nitroglycerin and calcium channel blockers (drugs that relax the coronary arteries so that more blood can flow through the narrowings), drugs that lower blood pressure, drugs that decrease cholesterol and blood fats. In addition to medicines, the patients will receive education of risk factors such as diet, exercise, and smoking. Recruitment for this study began June 1999 and patient follow-up will be to approximately June 2005.

MeSH terms: coronary disease; clinical outcomes.



# PRINCIPAL INVESTIGATOR:

Anne Sadler, R.N., Ph.D.

# PROJECT TITLE:

Sexual Victimization and the Military Environment: Contributing Factors, Vocational, Psychological and Medical Sequelae

#### **SUMMARY:**

In the United States violence is an occupational hazard for women. Violence is responsible for the deaths of more women in the workplace than from any other labor cause. In addition, up to one-quarter of women may experience rape in their lifetime. Military service is known to further increase women's risk of violence, yet the reason for this occurrence is unclear. The goal of this study is to determine military environmental factors associated with physical assault and sexual violence (harassment, sexu al touching, genital fondling, and attempted or completed rape) of service women, and to determine consequent vocational impairment, health status

impairment and psychological outcomes (such as post-traumatic stress disorder). Our preliminary data indicates that there are specific factors that predict physical assault and sexual violence towards service women. Current psychological functioning and health status of women veterans who were victimized during military service are negatively affected and result in higher rates of health care utilization. In military job performance, job satisfaction, and longevity of military service are also significantly impaired in service women who experienced physical assault or sexual violence in the military as compared to women who did not have these experiences. Such information could lead to the development and implementation of appropriate specific prevention strategies and could thereby directly improve the safety, health, and military effectiveness of service women.

MeSH terms: women; rape; PTSD; military environment; vocational impairment; health status.



#### PRINCIPAL INVESTIGATOR:

Larry S. Schlesinger, M.D.

#### PROJECT TITLE:

Biology of Tuberculosis and Macrophage Membrane Receptors

#### **SUMMARY:**

Tuberculosis is an enormous world health problem especially among the elderly and immuno-compromised patients such as those with AIDS. Three million people die from this respiratory disease annually. The efficacy of the BCG vaccine is highly variable and antibiotics are becoming less effective, in part because of new resistant bacteria. A goal of this work is to enhance our limited understanding of the host interaction with the causative bacterium, *Mycobacterium tuberculosis* (*M.tb*).

*M.tb* is transmitted from person to person by aerosolized droplet secretions that are inhaled into the small airways of the lung of an uninfected individual. Here *M.tb* is ingested by specialized blood cells called macrophages. Multiplying *M.tb* eventually destroys the macrophage, is disseminated throughout the body, and is ingested by other specialized macrophages in various organs. While multiplying within the macrophage, this cell transmits signals to other blood cells that are critical in establishing an effective host defense against this disease. Therefore, further defining the molecular basis of the *M.tb*-macrophage interaction is central to our understanding of tuberculosis.

Studies examine bacterium and host-derived molecules involved in M.tb ingestion and intracellular trafficking by human macrophages including lung macrophages. Macrophages containing *M.tb* and *M.tb* products will be examined for their influence on macrophage signaling by studying their effect on the synthesis and expression of normally produced macrophage products. This work will not only provide new critical information in understanding tuberculosis but may ultimately have great impact on the quality of patient care by pointing to new strategies for targeted immune therapy and vaccine development. Targeted therapy is expected to lead to less morbidity in treated tuberculosis patients and thus may have the added advantage of reducing the cost of treating such patients.

MeSH terms: infectious diseases; immunology; tuberculosis; macrophages; phagocytosis.



#### PRINCIPAL INVESTIGATOR:

Warren Schmidt, M.D., Ph.D. Douglas La Brecque, M.D.

# PROJECT TITLE:

A Multi-center Trial to Evaluate the Epidemiology, Natural History, and Treatment Response of Hepatitis C in the

# **United States Veterans Population**

# **SUMMARY:**

Hepatitis C is a virus that infects over 4 million people in the United States. The virus causes chronic infection and progressive liver disease in nearly 80% of infected patients and can lead to cirrhosis and liver cancer. As a group, veterans have a higher incidence of hepatitis C because of increased risks such as blood transfusions, battlefield exposure to blood, and illicit drug use. This is a multi-center study to evaluate risk factors for exposure to hepatitis C and the effectiveness of current treatment for hepatitis C in US Veterans. A total of 11,500 veterans are expected to be screened and evaluated in about 30 Veterans Administration Medical Centers across the United States. Approximately 2,500 patients will be enrolled into treatment. The duration of the study will be approximately 3 years during which each individual patient will be treated for 24-48 weeks and followed medically for a total of 72 weeks. Each veteran who is treated will be given standard combination antiviral therapy for hepatitis C, which is currently the recognized standard of care for patients with hepatitis C. Combination therapy consists of interferon given by injection three times weekly and oral ribavirion taken orally twice a day. In other large studies, combination therapy has been shown to be superior to interferon alone for long term, sustained remission from liver disease due to hepatitis C. The major goals of this large study are to: 1) collect and analyze data from veterans on the risk factors for exposure and transmission of hepatitis C and 2) to determine the effectiveness of combination therapy for treatment of hepatitis C in veterans.

MeSH terms: hepatitis C; chronic hepatitis; interferon; cirrhosis; liver disease.



PRINCIPAL INVESTIGATOR:

Warren Schmidt, M.D., Ph.D.

Jack Stapleton, M.D. Douglas La Brecque, M.D.

#### PROJECT TITLE:

Evaluation of Hepatitis Viruses in Peripheral Blood and Liver Biopsy Tissue of Individuals With Chronic Hepatitis

#### **SUMMARY:**

Chronic viral hepatitis is a liver disease caused by small viruses that are commonly referred to as hepatitis B, hepatitis C and, in some instances, hepatitis G. Hepatitis C and hepatitis B cause considerable liver disease worldwide and are major causes of chronic liver disease, cirrhosis, and liver cancer. For these reasons it is important to study how these viruses are carried in the blood stream and how they affect liver tissue. Although hepatitis G causes minimal liver disease, it is a virus with a similar structure and behavior to hepatitis C and is frequently found in individuals with hepatitis C or hepatitis B infections. Thus it is important to also study hepatitis G in humans. The purpose of this study is to examine patient blood and different blood cells for the presence of hepatitis viruses and to determine the effectiveness of anti-viral medications for decreasing or removing the viruses from the blood stream. It is also important to quantify the amount of virus in the blood to determine the body's response to therapy. A significant portion of this research also will evaluate the effects of the hepatitis viruses on liver tissue and determine the liver's response to the therapy. The goals of these studies are to 1) determine how the virus is carried in the bloodstream 2) investigate how the virus from the blood interacts with the liver to produce liver disease and 3) to quantify the response of blood and tissue levels of virus with anti-viral treatments.

MeSH terms: hepatitis C; hepatitis G; chronic hepatitis; cirrhosis; liver disease.



#### PRINCIPAL INVESTIGATOR:

Konrad Schulze, M.D., FRCP

#### PROJECT TITLE:

**Mechanics of Stomach and Small Intestine** 

# **SUMMARY:**

The stomach is important in storing food and preparing for uptake by the body. In the process it enlarges, it breaks down food and empties it slowly and steadily. When the stomach does not function well in these processes, discomfort, heartburn, indigestion, nausea, weight loss and difficulties with the body's fuel homeostasis occur. This is particularly common after operations on the stomach and in diabetes mellitus.

Currently, it is unclear how the stomach accomplishes these functions. We have used modern imaging techniques to analyze how the movements of the stomach interact with its contents. We are particularly interested in how these interactions are influenced by the gastric sling fibers, a specialized muscle bundle which joins the dependent part of the stomach to the food pipe and might regulate flow inside the stomach by changing its shape and the distance to the gastric outflow.

MeSH terms: muscle, smooth; colonic haustra; intestinal flow; obstruction.



# PRINCIPAL INVESTIGATOR:

William I. Sivitz, M.D.

# PROJECT TITLE:

Hormonal and Metabolic Regulation of Glucose Transport and GLUT-1 Expression in Endocrine Responsive Breast Cancer Cells

#### **SUMMARY:**

My laboratory has been studying mechanisms controlling glucose use by cells for the past ten years. In particular, we have investigated the function of glucose transporter molecules that bring glucose into cells.

In this research we have studied the means by which breast cancer cells use glucose. We found that a particular type of transporter molecule called GLUT-1 was largely responsible for glucose uptake into breast cancer cells. We also found that more aggressive breast cancer cells with a higher tendency to metastasize expressed more of the GLUT-1 transporter molecules. Cells that expressed more GLUT-1 tended to grow faster. Also, mammary (breast) tumor tissue of live rats contained high levels of GLUT-1 within the neoplastic cells.

If we can understand mechanisms that regulate glucose use, we may be able to intervene to specifically alter glucose use by tumor cells, but not by normal cells. If we can specifically determine what controls the activity of GLUT-1, we may be able to alter this activity within breast cancer cells and slow or prevent their growth.

MeSH terms: breast cancer; glucose; glucose transport.



# PRINCIPAL INVESTIGATOR:

William I. Sivitz, M.D.

#### PROJECT TITLE:

Metabolic Interaction of Insulin and Leptin and the Regulation of Nerve Activity and Vascular Reactivity

**SUMMARY:** 

Recently a protein called leptin has been discovered in mice and humans which appears important in regulating body weight. Leptin is produced by body fat cells. There is strong evidence that leptin is secreted as part of a mechanism by which body fat mass is regulated. Whenever food intake is abundant and weight increases, fat tissues secrete leptin. The body responds to this by increasing its metabolic rate and by decreasing appetite. During periods of deficient food intake the opposite occurs; leptin levels decrease which decreases metabolic rate and increases appetite. Hence, the protein appears to function as part of a feedback mechanism of regulating body fat mass. Insulin and glucose also have profound effects on body fat stores and metabolism. Therefore, it is important to identify interactions between glucose and insulin and leptin. This will improve our understanding of diabetes and likely enable means of controlling body weight, which is extremely important to the control of diabetes.

In this research, we have found that blood leptin concentrations and leptin production by fat cells is rapidly and markedly increased by insulin. This effect is independent of concurrent changes in body weight or glucose. Blood leptin concentrations and leptin production are markedly reduced in insulin deficient diabetic rats. We have also found that leptin treatment of rats rapidly increases nerve activity in sympathetic nerves by triggering nerve activity in the central nervous system. This nerve activity may be critical to the effect of leptin to regulate body fat mass and possibly for additional effects of leptin potentially including blood pressure regulation. We also found that acute administration of leptin increases the response of body tissues to insulin. Also, we have found that leptin and food intake alters body proteins called "uncoupling proteins" that control heat production. This effect of leptin could also play an important role in the action of leptin on body weight. We continue to investigate the way in which leptin might control metabolism, nerve activity, and blood vessel function.

MeSH terms: obesity; diabetes; glucose; leptin; insulin.



PROJECT TITLE:
Hepatitis C Projects

# **SUMMARY:**

There are several different studies of hepatitis C virus (HCV) ongoing in this laboratory. HCV persistently infects approximately 4 million Americans, and is the leading cause of disease leading to liver transplantation in the U.S. Following many years of HCV infection (usually around 20 years), cirrhosis in about 20% of infected people. A variety of diseases outside of the liver are also caused by hepatitis C, including a kidney and rheumatological diseases. Although blood exposure is the major means of HCV transmission, the mode of transmission is unknown in up to half of infected people. Furthermore, there is an increased rate of infection in Veterans and alcoholics. A variety of studies indicate that HCV and alcohol are synergistic in causing liver disease. The mode of transmission of HCV in alcoholics is not clear, and the way in which alcohol and HCV enhance liver disease is not well understood. Our laboratory has four major areas of HCV research. First, we are investigating the epidemiology of HCV infection in alcoholic liver disease to better understand transmission factors and the relationship between HCV and alcohol use. Related to this we are studying the effects of ethanol on HCV gene regulation and cellular interactions. Secondly, we are applying a wholeblood based HCV diagnostic test we developed to the study of alcoholic liver disease. In addition, we are studying the mechanism by which the virus in some individuals evades detection by the standard HCV antibody testing systems. To do this, we are studying both virologic features of viruses found in "antibody negative" people, and studying the immune response in these individuals to "standard" HCV proteins, and HCV proteins from isolates found in these individuals. Thirdly, we are investigating

how HCV binds to and enters susceptible cells. The purpose of this work is to identify potential targets for treatment and/or vaccination, and to better understand how HCV causes liver, kidney and rheumatologic disease. Finally, we have developed an infectious clone of a closely related flavivirus called GB virus type C (GBV-C). This clone grows in CD4+ T cells, and we are studying this system as a model for HCV infection. Since HCV does not grow well in cell culture, these studies and the virus binding and entry studies may help to develop a "test-tube" system for studying hepatitis C virus. Finally, since GBV-C causea life-long infection in humans and does not appear to cause any disease, it could potentially serve as a gene therapy vector. Studies are underway to test the feasibility of using the GBV-C infectious clone to express foreign genes.

MeSH terms: hepatitis C; HCV; alcohol virology; RNA sequence; receptor.



PRINCIPAL INVESTIGATOR: Jack Stapleton, M.D.

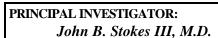
PROJECT TITLE: HIV Studies

### **SUMMARY:**

There are a variety of clinical and basic science studies on HIV ongoing in this laboratory. At the VA laboratory, we have developed a practical method for quantifying HIV replication in T cell lines, and have applied this to compounds that theoretically might have anti-HIV activity. We have identified new inhibitors of cellular activation which have significant anti-HIV activity, and which have been shown to be well tolerated by humans. In addition, a variety of antibacterial peptides have been shown to have broad spectrum antiviral activity. These compounds are being further evaluated to determine the mechanism of action, and potential for application in human studies. Collaborations with immunologists and cell biologists are also

ongoing. These studies are designed to study the effect of immune modulation by nucleic acis molecules or ethanol on HIV growth in the test tube. Collaborative studies of HIV interactions with T cells and the effects of cell fusion on viral replication and spread are also ongoing with faculty from the University of Iowa Biology Department. In association with the University of Iowa clinical HIV program and the subunit of the NIH AIDS Clinical Trials Groups, interested patients at the Iowa City VA are referred to the ACTG. Trials ongoing include IL-2 plus highly active antiretroviral therapy (HAART), HIV gp120 vaccination plus IL-2 and HAART, protease-sparing drug combination studies, and a macrophage modulator (WF-10). It is anticipated that some of the compounds identified in the VA in vitro studies will be applied to patients in this program.

MeSH terms: NIV; AIDS; alcohol; human immunodeficiency virus; clinical trials.



PROJECT TITLE:

**Mechanisms of Steroid Hormone Effects on Ion Transport** 

#### **SUMMARY:**

The VA funded research conducted in my laboratory involves the understanding of how Na and C1 ions are eliminated in the urine. Each day the salt (Na and C1) we eat must be eliminated by the kidney. Failure of the kidney to regulate NaC1 excretion with great precision will result in hypertension or accumulation of fluid. Na and C1 are filtered by special kidney filters called "glomeruli" and most of the NaC1 is absorbed by cells along the functioning units of the kidney. The fine tuning of NaC1 excretion occurs by regulation of specific ion channels on cell membranes. A variety of hormones and physical factors interact to regulate the transfer of these

ions. We are developing evidence that some forms of hypertension result from abnormal regulation of these processes. The ability to understand how ion channels function has already resulted in identifying some genetic causes of high blood pressure. Further understanding of ion channel regulation will provide important clues for further advances into specific treatments of high blood pressure and prevention of cardiovascular disease.

MeSH terms: ion transport; NaCl excretion; steroid hormones; nephrology.



William T. Talman, M.D.

PROJECT TITLE

Cardiovascular Responses to Glutamate Linked to Nitric Oxide

# **SUMMARY:**

The nucleus tractus solitarii (NTS) plays a critical role in cardiovascular regulation. Glutamate (GLU) is a putative neurotransmitter in cardiovascular regions of the NTS where nitric oxide (NO·) or nitrosyl factors elicit cardiovascular responses similar to those produced by GLU. Activation of GLU receptors at other central sites has been shown to cause release of NO. but studies have not been done to determine if GLU influences NO· in NTS and, if so, if NO· contributes to responses to GLU. Preliminary data suggest that GLU and NOcontaining nerves lie in close proximity in NTS and that inhibition of synthesis of NO· by local neurons attenuates responses to GLU. The hypothesis of the current study is that responses to GLU in NTS are linked to NO. There are four short-term objectives. The first is to determine if

NO participates in mediating cardiovascular effects produced by GLU receptor agonists in the NTS. The second aim is to determine if NO· is released from the NTS exposed to GLU receptor agonists. The third aim is to determine if effects of GLU on soluble guanylate cyclase are mediated through NO. The fourth aim is to determine the anatomical relationship between GLU terminals and neuronal elements that contain NO· synthase (NOS) in the NTS. Our studies will use pharmacological, immunohistochemical, immunoelectron microscopic, neurochemical, and physiological approaches to investigate a link between the two putative transmitter mechanisms. Adult rats will be used in all studies. Pharmacological studies will assess cardiovascular responses to injection of GLU agonists into the NTS of awake. unrestrained rats both before and after injection at the same site of reduced hemoglobin, a scavenger of NO:; an inhibitor of neuronal NO: synthase; L-arginine, the precursor of NO:; a donor of NO; or an inhibitor of soluble guanylate cyclase. The in vitro study of NO· release will utilize freshly prepared tissue from NTS dis sected from slices of rat brain stem and will measure NO. by the chemiluminescence method. Another in vitro study will seek immunohistochemical evidence linking neuronal elements that contain both glutamate and nitric oxide synthase. While the studies do not directly address clinical issues, they may show a potentially important relationship between glutamatergic and nitroxidergic systems in the NTS. The possible interactions between these systems could impact on the vital role played by the NTS in regulation of blood pressure, ventilation, and gastric motility. The studies therefore pave the way for investigations of derangement in these transmitter mechanisms in the presence of diseases affecting the particular organ systems.

MeSH terms: neuropharmacology; nitric oxide; baroreceptors; reflex; neurotransmitters; glutamic acid; immunohistochemistry solitary nucleus.



PRINCIPAL INVESTIGATOR

William T. Talman, M.D.

# PROJECT TITLE

Pathways Connecting Cardiovascular Regions of the Nucleus Tractus Solitarii with the Superior Salivatory Nucleus

# **SUMMARY:**

The arterial baroreceptor reflex plays a critical role in regulating regional blood flow to peripheral organs. Our own studies suggest that the reflex may also participate in regulating the tone of cerebral blood vessels and thus cerebral circulation. Interruption of arterial baroreceptor nerves significantly blunts cerebral vascular dilatation, known as breakthrough, that occurs when the cerebral circulation is exposed to very high blood pressures. Breakthrough is likewise attenuated by central disruption of the baroreflex effected by blocking function of the nucleus tractus solitarii, the site of termination of baroreceptor afferent nerves; by blocking synthesis of nitric oxide; or by interrupting postganglionic parasympathetic nerve fibers that release nitric oxide on cerebral arteries. Because of these background findings we hypothesize that arterial baroreceptors are connected to preganglionic parasympathetic neurons that modulate cerebrovascular resistance via a pathway from the nucleus tractus solitarii. We propose to define the anatomic pathways underlying baroreceptor influences on parasympathetic innervation of cerebral vessels. These studies will utilize chemicals that are transported to and from nerve cells in the brain and can be visualized in the brain after their transport. Our studies will identify projections from the nucleus tractus solitarii to the superior salivatory nucleus, the site from which nerves project from the central nervous system to peripheral parasympathetic nerve cells. The studies will, furthermore, topographically map the superior salivatory nucleus with respect to sites of origin of functionally specific preganglionic axons and they will define relationships between those sites and sites where projections from the nucleus tractus solitarii terminate. The studies will clarify a potentially important neural mechanism for regulating circulation in the brain. A pathway that could be involved in allowing the brain to control its own blood flow and potentially to

protect itself during periods of ischemic stress will likely be the most interesting aspect of our studies to the general public.

MeSH terms: baroreceptor reflex; blood pressure; cerebrovascular circulation; hypertension; neurology; neurotransmitters; nitric oxide; parasympathetic nervous system; pons; solitary nucleus



PRINCIPAL INVESTIGATOR:

Lubomir P. Turek, M.D.

PROJECT TITLE:

**Human Papillomavirus - 16 Gene Regulation in Cervical Cancer** 

#### **SUMMARY:**

Cervical cancer is a common type of cancer in women, and although it can often be detected early by regular checkups, a number of aggressive cases that apparently escape early detection are diagnosed every year. Cervical cancer is unusual in that it is most often caused by infection with a virus, one of several types of human papillomaviruses (HPVs). HPV type 16 is the most common strain found in ~40-50% of all cervical cancers. In many cases, only disrupted fragments of the viral genome are found integrated in the DNA of the cancer cells. Our projects focus on the molecular biology of papillomavirus replication and the regulation of viral gene expression. Until recently, studies on HPV were limited because the viruses do not grow in cells in the laboratory. Together with our collaborators, we have developed an assay for HPV-16 replication (growth) that permits us to test the role of different viral genes and cellular regulatory factors on the different stages of HPV infection, and will determine under what conditions virus replication is disrupted and results in the integration of viral fragments in the genome of the cell. The experiments will provide information that will improve the understanding of HPV infection and its role in cancer, and

possibly aid in improving diagnosis and therapy in the future.

MeSH Terms: papillomavirus; cervical cancer; gene regulation; pathology.



#### PRINCIPAL INVESTIGATOR:

Bonnie Wakefield, Ph.D., R.N. James Flanagan, M.D., Ph.D.

#### PROJECT TITLE:

Implementation and Evaluation of Telemedicine to Improve Health Care

# **SUMMARY:**

Telehealth has the potential to enhance patient care by increasing access to specialized and primary health care and decreasing costs. Research on telehealth must focus on is sues surrounding integration of the technology into the existing organizational and professional culture and evaluating its effectiveness in improving outcomes and decreasing costs. The goal of this program is implementation and evaluation of specific telemedicine applications. Subjects include patients and providers at the Iowa Veterans Home and providers at the Iowa City and Des Moines VA Medical Centers. The outcome of the consultation (admission, clinic appointment, change in treatment), length of the consult, equipment used, and qualifications of the health care provider provides data for cost analyses. Patient and provider satisfaction are also evaluated. Results from each evaluation are as follows:

Chronic Wound Consultation Clinic. Inter-rater reliability for nine different wound characteristics ranged from 54 to 100%. The cost of telehealth wound consultations was estimated to be \$92.80 for each 20-minute consultation. The long-term care residents found the telehealth consultation to be as good as the in-person assessment, although patients noted difficulty hearing and

seeing the telehealth consultant. Nurses were equally satisfied with both the telehealth and inperson consultations and felt both consultation modes were a productive use of their time and skills.

Urgent Care Clinic. Procedures were developed

and pilot-tested. Even though patients were quite favorable to the technology, IVH physicians have not used telemedicine for Urgent Care referrals, even though there is potential for cost avoidance for transportation. Telemedicine Specialty Clinics. As of December 1999, 75 individual specialty clinic consultations for IVH residents have been completed. Most of these have been follow up visits. The most frequently used clinics have been GU (n=20), neurology (n=21), cardiology (n=12), and general surgery (n=10). Physicians ratings are: 76% good to excellent for usefulness in developing a diagnosis; 85% good to excellent for usefulness in developing a treatment plan; 75% good to excellent quality of transmission; and 84% good to excellent satisfaction with the consult format. Overall, 81% of patients were satisfied with the consult format, while 6% were neutral and only 4% were somewhat dissatisfied (10% did not respond to this question). For the VA health care system, and the public at large, telehealth has the potential to increase access to specialized care provided by physicians and other health professionals, enhance training of health professions students, and provide patient and staff education. Research such as this project will help determine under what conditions and for which types of patients telehealth will be most applicable and cost-effective.

MeSH Terms: telemedicine; long-term care; wound healing.



### PRINCIPAL INVESTIGATOR:

Bonnie Wakefield, Ph.D., RN Linda Diggelmann, MT (ASCP) Janet Mentes, RN, GNP, ABD Ken Culp, Ph.D., RN

PROJECT TITLE:

# Predicting Impending Dehydration in Elderly Veterans

# **SUMMARY:**

Dehydration is the most common fluid and electrolyte disorder in long-term care residents and is associated with geriatric syndromes such as delirium, falls, and incontinence. The existing literature describes clinical and laboratory signs of actual dehydration, yet indicators of impending or mild dehydration are less wellstudied. Clinicians need low cost, practical clinical indicators of impending or mild dehydration in order to institute treatment and prevent progression to more severe dehydration. This study proposes to validate a low-cost practical method to detect impending dehydration and to assess its' utility as a risk indicator to predict dehydration. Specifically, we will determine whether urine color as measured by a urine color chart accurately reflects hydration status as measured by urine specific gravity and osmolality. Specific hypotheses include: 1) increasing scores as measured by the urine color chart are associated with increased urine specific gravity and increased urine osmolality in elderly veterans; 2) low to moderate scores (1-6) on the urine color chart, urine specific gravity, and urine osmolality are not associated with serum sodium, BUN/creatinine ratios, and serum osmolality; and 3) high scores (7-8) on the urine color chart, urine specific gravity, and urine osmolality are associated with serum sodium, BUN/creatinine ratios, and serum osmolality. Use of the color chart to measure hydration status as measured by urine and serum/plasma indicators was found to be valid in younger athletes (Armstrong, et al., 1994); we will test whether this association is valid in older adults. The sample includes 89 hospitalized patients and long-term care residents. Urine samples were collected and analyzed at baseline and after 10 hours of fasting. Data were collected on food intake and medications. Preliminary results show a significant positive correlation between urine color and urine specific gravity and osmolality ( $p \le 0.01$ ); and significant differences between baseline and fasting urine osmolality (t = -1.98,  $p \le 0.05$ ) and between baseline and fasting urine specific gravity (t = -3.11, p<0.01). Using the risk appraisal of

impending dehydration, future larger studies will conduct a clinical trial of an intervention, the hydration management guideline, to prevent dehydration and it's associated adverse outcomes in elderly veterans in acute and long term care settings. Nurses are in a unique position to conduct assessments to predict and prevent this potential negative syndrome. Using practical tools such as the urine color chart and a specific risk assessment process to detect impending dehydration, treatment can be instituted early to prevent actual dehydration. Use of a targeted assessment may also reduce nursing time, eliminating routine time-consuming assessments of all patients, e.g. 24-hour intake and output records, encourage more appropriate focused efforts on at-risk patients. Use of clinical guidelines is becoming increasingly accepted within health care and important in the VA as a whole to improve appropriateness of care and efficient use of resources. Evaluation of the guideline in future studies through a rigorous clinical trial will enable efficient, appropriate, and high-quality patient care.

MeSH Terms: dehydration; urine; aged.



# PRINCIPAL INVESTIGATOR:

Bonnie Wakefield, Ph.D., R.N.

# PROJECT TITLE:

Preventing Functional Decline in Hospitalized Elderly

#### **SUMMARY:**

The project proposes Phase I of a multi-phase research agenda that will examine nursing interventions to maintain, improve, or prevent deterioration in functional status in hospitalized patients, determine effective staff development mechanisms to care for at-risk elderly hospitalized patients, and examine use of evidence-based guidelines to ensure cost-effective and optimal continuity of care for high risk frail elderly. The

goal is to develop a better understanding of the natural history, clinical profiles, and trajectories of functional decline in hospitalized elderly veterans (HEV), and clinical and resource importance in HEV. Risk profiles will be developed so that evidence-based interventions to prevent and treat this phenomenon can be developed and tested. The immediate objective of the proposed study is to describe patterns and trajectories of functional decline associated with different chronic illnesses and comorbid conditions. The long term objectives of this program of research are to test evidence-based guidelines and identify the organizational structures necessary to support care for high risk frail elderly both in the hospital and after discharge. Specific Aim 1: Characterize the natural history and trajectory of functional decline associated with hospitalization in elderly veterans. Research questions to be addressed are: 1) What is the prevalence and type of functional limitations present on admission to the hospital?' 2) What is the incidence of functional decline in hospitalized elderly?; and 3) What is the nature, magnitude, and trajectory of change in functional status during hospitalization? Specific Aim 2: Identification of the subset of patients who are at high risk for decline during hospitalization. The primary research question to be addressed in this aim is: Can subgroups of hospitalized patients who experience decline and increased resource use after hospitalization be identified using a combination of risk factors? Research questions to be addressed are: 4) What are the risk factors for functional decline during hospitalization? And 5) Is functional decline during hospitalization associated with new admission to a long term care setting?

MeSH Terms: activities of daily living; aged; nursing assessment.



PRINCIPAL INVESTIGATOR: Michael Wall, M.D.

PROJECT TITLE:

Motion Perimetry: A New Method for Early Detection of Visual Loss

# **SUMMARY:**

It is well known that diseases of vision can result in detectable visual loss that goes unnoticed by the patient. Because of the lack of effective screening tests, patients with these diseases can be misdiagnosed or go undiagnosed until they have developed substantial visual loss. Visual field testing (perimetry), is our most important test for detecting visual system damage. Although used for over 140 years, it remains time consuming and tedious for the subject. Conventional perimetry also lacks sensitivity and therefore has never become practical as a clinical screening tool. In addition, it is poorly designed ergonomically. This leads to patient discomfort during testing and test-induced artifacts.

The aim of this research is to develop a practical screening test to detect visual loss at an earlier stage. We are applying recent scientific advances with computer graphics technology to develop a new test—Motion Perimetry. Our results to date demonstrate that the test has a high sensitivity to detect disease. In addition, Motion Perimetry is ergonomically designed with feedback and video game-like rewards that have improved patient acceptability. Since Motion Perimetry is specifically designed to test variables that should change with damage to visual neural networks (the brain's interconnected wiring), we are also gaining knowledge that is relevant to visual information processing. We are currently developing a modification — eye movement motion perimetry — that tests vision by having patients look at visual stimuli while recording their eye movements using a video camera.

Motion perimetry has: (1) an increased sensitivity for detection of visual system damage, (2) improved test reproducibility, (3) a greater relationship with performance of everyday tasks and (4) provides important information about how visual information is processed.

MeSH Terms: visual testing; perimetry; motion perception; glaucoma; visual field.

PRINCIPAL INVESTIGATOR: Jerrold Weiss, Ph.D.

PROJECT TITLE:

Neutrophils and Bacterial Phospholipid Degradation

#### **SUMMARY:**

This project concerns further studies on the role and regulation of the action of mammalian Group IIA phospholipase A2 (PLA2) during inflammation and the determinants of the degradation of Gram-negative bacterial lipopolysaccharides during host antibacterial action. Most the manifestations of infectious diseases caused by bacteria reflect either the directly toxic actions of specific bacterial products or dysfunctional actions of the host in response to bacterial products. Thus, effective host defense requires not only sequestration and elimination of viable invading microbes but also digestion, disassembly and detoxification of microbial products. Our focus on digestion of bacterial phospholipids and glycolipids (i.e. lipopolysaccharides; endotoxin) reflect the essential role of these bacterial products in maintaining the structural integrity of bacteria and in provoking both protective but also potentially life-threatening host responses to infection. Recent studies have established that Goup IIA PLA2 is a major extracellular weapon against invading bacteria, especially Grampositive bacteria. The antibacterial activity of this enzyme is a property that is unique to this isoform and not shared by any of the other closely related PLA2. Conversely, the toxic properties of related venom PLA2 toward human cells is not exhibited by this host enzyme. Thus, a major focus of our work is directed to achieving a better understanding of the molecular determinants of the antibacterial action of the mammalian Group IIA PLA2 in particular, and of

the target cell selectivity of this family of enzymes more generally. In the long term, these studies are directed toward addressing two fundamental questions: (1) What determines the extent of bacterial digestion and disassembly during host antibacterial action? (2) What regulates the action of defined phospholipases on the phospholipids of biological membranes? These studies are likely to provide new insights related to the mechanisms of host defenses in infection and of the function of membranes in general. In addition, the potency of the Group IIA PLA2 against clinically important bacterial pathogens, including multi-drug resistant strains, and this enzyme's ability to act in biological fluids raises the possibility that these studies may lead to development of novel antibacterial agents needed when host defenses are limited and conventional antibiotics are no longer effective.

MeSH terms: gram-negative bacteria; grampositive bacteria; neutrophils; inflammation; endotoxin; phospholipids; bactericidal; phospholipases; deacylases; membranes; staphylococcus aureus; antibiotics.

PRINCIPAL INVESTIGATOR: Jerrold Weiss, Ph.D.

PROJECT TITLE:

Microbicidal Activity of Leukocytes: Active Factors

### **SUMMARY:**

This project concerns the further exploration of the mechanisms of host response and defense against invading gram-negative bacteria. A major focus is the molecular and functional characterization of the bactericidal/permeabilityincreasing protein (BPI) and of cathelicidinrelated proteins, lipopoysaccharide (LPS)-binding antimicrobial proteins from polymorphonuclear leukocytes. These proteins are studied both as isolated proteins and as mobilized components of PMN-rich inflammatory exudates that closely mimic host response to infection. These proteins have been discovered, isolated and cloned in our laboratory and implicated (BPI, especially) as important intracellular and extracellular cytotoxins against gram-negative bacteria and negative regulators of host responses to LPS (endotoxin). Insights gained from this work are expected to provide a better understanding of host response to endotoxin and defense against invading gramnegative bacteria. They may also contribute to the design and development of new therapeutic agents against invasive gram-negative bacterial infections and endotoxemia that are needed when host defenses of patients and conventional antibiotics are inadequate. A recombinant derivative of BPI is in advanced stages of clinical testing, a direct outgrowth of fundamental studies originally carried out in our laboratory.

MeSH terms: gram-negative bacteria; neutrophils; inflammation; endotoxin; sepsis; bactericidal; phagocytosis; antibiotics.

# PRINCIPAL INVESTIGATOR:

Jerrold Weiss, Ph.D.

# PROJECT TITLE:

Mobilization and Delivery of Meningococcal Lipoligosaccharides to Host Targets

# **SUMMARY:**

Host responses to lipopolysacchrides (LPS) or lipooligosaccharides (LOS) of Gram-negative bacteria are believed to play a major role in defense against many invasive Gram-negative bacterial infections. These normally protective responses can also cause life-threatening systemic inflammatory disorders when

inadequately controlled. This is dramatically illustrated in fulminant meningococcal septicemia, an extremely acute disease that appears linked to the accumulation of extraordinarily high levels of LOS. The broad long-term objectives of this project are to better understand the molecular determinants of LPS/LOS (endotoxin) mobilization and its interactions with defined host targets. A major focus is on the mechanisms of biosynthesis, assembly and dissemination of released bacterial membrane "blebs", believed to represent an important form of disseminated endotoxin in vivo and a particularly prominent feature of growing Neisseria meningitidis in vitro. These studies are likely to provide new insights concerning two fundamental questions: (1) What controls the mobilization of endotoxin during bacterial interaction with the host? (2) How do the unique physical characteristics of meningococcal LOS and of cell-free LOS-containing membrane fragments affect the interaction of endotoxin with host machinery that mediate either pro- or antiinflammatory responses?

MeSH terms: lipooligosaccharides; neisseria meningitidis; inflammation; endotoxin; endotoxin-binding proteins; capsule; deacylases; outer membrane; lipoproteins.



PRINCIPAL INVESTIGATOR: Mary E. Wilson, M.D.

PROJECT TITLE:

Interactions of *Leishmania*Chagasi with Host Macrophages

#### **SUMMARY:**

Leishmaniasis refers to a group of diseases caused by parasitic protozoa belonging to the genus *Leishmania*. The disease assumes different forms depending on the particular parasite species involved, ranging from a chronic skin ulcer to a fatal visceral disease. It is

acquired after the bite of an infected sandfly in endemic regions such as the Middle East, countries bordering the Mediterranean Sea, South and Central America, and Africa. A new form of the leishmaniasis has recently been described in personnel returning from the Persian Gulf conflict. Despite its importance to residents of endemic countries and to American military personnel in the Middle East and Central America, the modalities for treatment of leishmaniasis remain suboptimal and there is not an approved vaccine.

This project is designed to study biochemical characteristics of the parasite that enable it to successfully survive in humans, and changes that occur in human cells after invasion of the parasite. These characteristics could be important in developing new forms of treatment for the disease. Our laboratory also studies parasite antigens against which humans are able to mount an effective immune response. The latter may eventually be useful in development of a protective vaccine. We have the ability to cultivate all species of *Leishmania* in our laboratory, and have successfully isolated Leishmania major from individuals who acquired leishmaniasis in the Persian Gulf and in Central America. The availability of a laboratory able to work directly with human Leishmania isolates and clinical expertise on this important pathogen provides us with a unique contribution to the evaluation of our VA patients.

MeSH terms: infectious diseases; immunology; Persian Gulf; leishmaniasis; parasitology; vaccine; treatment; *Leishmania*; protozoa.



PRINCIPAL INVESTIGATOR:

Patricia L. Winokur, M.D.

PROJECT TITLE:

Analysis of Human Papillomavirus E2 Protein Interaction

#### **SUMMARY:**

Human papillomaviruses induce tumors of the skin, respiratory tract and genital tissues. These tumors range from benign skin warts to invasive cancers of the cervix and other tissues. Genital warts are the most common viral sexually transmitted disease today and cervical cancer is the second most common cancer of women throughout the world.

The E2 protein from papillomaviruses plays a major role in many viral functions. If the E2 protein is damaged, the abilities of the papillomavirus to make messenger RNA, replicate itself and transform host tissues into cancer-like cells are disrupted. The E2 protein is divided into two sections, one half that binds DNA and the second half that is absolutely crucial for messenger RNA production and replication. We hypothesize that this second half of the protein function through various protein-protein interactions with other viral and/or cellular proteins to accomplish these different tasks. The HPV 11 E2 protein has been purified and used to evaluate binding between E2 and replication protein A, a cellular replication factor thought to be important in replication of other DNA viruses. We were unable to detect any binding between the two proteins. Next this purified E2 protein was used to screen a random peptide phage display library. Over 100 phage that bound to E2 were sequenced. Several interesting peptide motifs were identified. One motif mapped to a region on the papillomavirus E1 protein which, along with E2, is required for papillomavirus replication. A second motif was identified that showed a strongly basic charge and within this strongly charged motif there appeared to be a particular sequence that corresponds to a cellular protein, ATF2. Studies are underway analyzing the ability of E2 to bind E1 and ATF2 and whether peptide sequences that correspond to the binding motifs can interfere with viral messenger RNA production or replication. Additionally a large panel of E2 mutants will be used to try to map the binding sites between these proteins. These studies could identify peptides that could be used to create a papillomavirus-specific antiviral therapy or structures that can be used to create antiviral molecules.

MeSH terms: viruses; virus replication; papillomavirus; transcription, genetic.



# PRINCIPAL INVESTIGATOR: Catherine Woodman, M.D.

#### PROJECT TITLE:

The Relationship Between Fibromyalgia and Psychiatric Disorders in Persian Gulf War Veterans: A Family Study

#### **SUMMARY:**

Fibromyalgia is a frequent diagnosis among Persian Gulf War veterans. Previous studies in other populations suggest that fibromyalgia cooccurs with psychiatric disorders, which are also common in the group of veterans of the Persian Gulf War. The purpose of this study is to use family study methodology to examine whether fibromyalgia is a familial illness in Persian Gulf War veterans. The overarching hypothesis is that there is a familial association between fibromyalgia and depression and anxiety disorders. This association may be due to genetic or environmental factors. Based on this hypothesis, we predict that fibromyalgia and depression and anxiety disorders will co-occur (i.e. relatives of probands with fibromyalgia will have fibromyalgia, relatives of probands with a psychiatric disorder will have a psychiatric disorder, and relatives of probands with fibromyalgia and a psychiatric disorder will have both fibromyalgia and a psychiatric disorder). This does not imply that we believe that a single gene is responsible for each disorder and that these genes are linked. Rather, we believe that the clinical definition of these disorders overlap and need further clarification. A secondary purpose is to study the relationship between fibromyalgia and psychiatric disorders within families. We hypothesize that fibromvalgia will co-occur with psychiatric disorders in the relatives of veterans with fibromyalgia and

provide evidence of a true relationship between the disorders. The study will be conducted in a population of veterans from the Persian Gulf War. First degree relatives (parents, siblings and adult children) of 75 subjects with fibromyalgia and 75 control subjects will be personally interviewed and examined to identify fibromyalgia and psychiatric disorders. The study will follow established procedures for family interview studies and employ state-of-the-art instruments of proven sensitivity to screen for the disorders under study.

MeSH terms: Persian Gulf War; veterans; stress; fibromyalgia; genetics.



PRINCIPAL INVESTIGATOR: Mark A. Yorek, Ph.D.

#### PROJECT TITLE:

Circulating Factors in the Etiology of Diabetic Vascular Disease

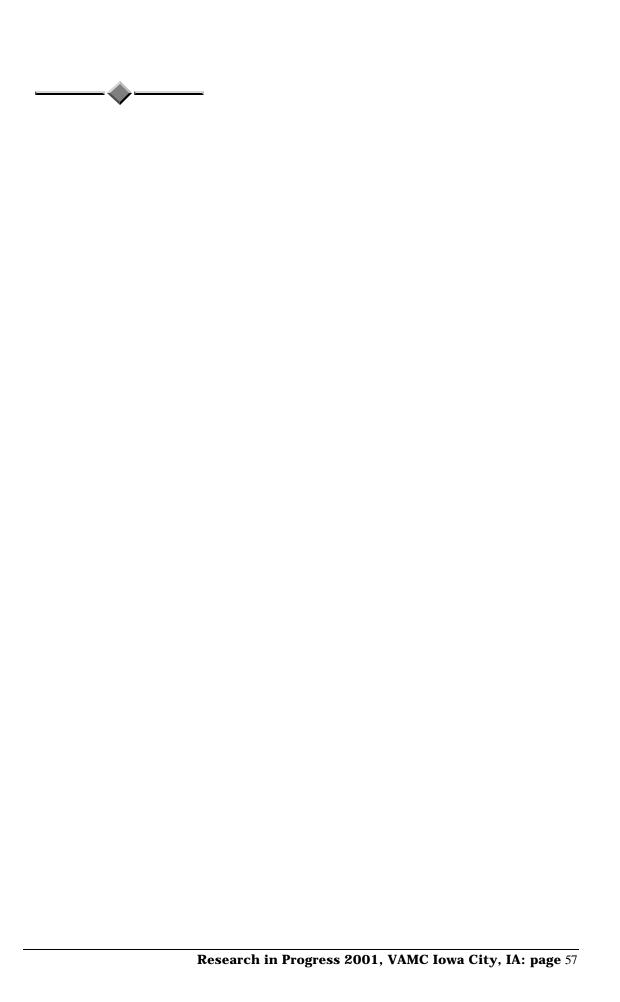
#### **SUMMARY:**

The goal of this research project is to improve our understanding of the molecular regulation of the sodium-dependent myo-inositol cotransporter (SMIT) in diabetes and other physiological conditions. Myo-inositol is an important component of phosphoinositides that have a role in signal transduction pathways, changes in intracellular myo-inositol levels also contributes to osmotic regulation. In diabetes, reduced levels of myo-inositol in the peripheral nerve has been linked to the development of diabetic neuropathy. It is believed that hyperglycemia in diabetes mellitus is responsible for altering myo-inositol transport, thereby leading to reduced myo-inositol levels. However, the mechanism responsible for this effect is unknown. Studies being conducted in my laboratory have been investigating the effect of hyperglycemia and increased levels of another monosaccharide, L-fucose, on myo-inositol

metabolism in cultured neural and endothelial cells; and in animal models, the effect of elevated L-fucose levels on vascular and neural function. Currently, the role of L-fucose in diabetes-related vascular and neural defects is under investigation.

Previously, we have shown that increased levels of L-fucose causes a competitive inhibition of myo-inositol transport by cultured endothelial and neural cells. This contrasts to the effect of hyperglycemia which has been shown to be a competitive inhibitor of the SMIT, but with a much higher K<sub>m</sub> than L-fucose, as well as a noncompetitive inhibitor, through the production of sorbitol which causes a decrease in SMIT mRNA. Aldose reductase inhibitors prevent this latter effect. L-fucose is a monosaccharide that we have shown is increased in the circulation of diabetic patients. Our studies have shown that increasing L-fucose levels in rats by supplementing the diet with 20% L-fucose for sixweeks, caused an increase in serum L-fucose levels that are similar to the levels of L-fucose in diabetic patients. This resulted in a decrease in motor conduction velocity, endoneuril blood flow and sciatic nerve Na<sup>+</sup>/K<sup>+</sup>ATPase activity and myo-inositol levels. These changes were similar to those that occur in diabetic rats. Other studies have shown that chronic L-fucose elevations for six months caused morphological changes in the sural nerve that were also similar to diabetic rats. These changes were partially prevented by supplementing the diets with myo-inositol or by treatment with acetyl-L-carnitine. These results suggest that other monosaccharides in diabetic circulation may contribute to neural and vascular defects and that a better understanding of these conditions is needed in order to design treatments to prevent diabetic complications affecting the nerve and vascular tissue. Current studies are focusing on the role of L-fucose in diabetic neuropathy and vascular disease in laboratory rats and the levels of free and proteinbound L-fucose in diabetic patients.

MeSH terms: endocrinology; metabolism; biochemistry; diabetes; diabetic neuropathy; diabetic vascular disease; L-fucose; hyperglycemia.



# **Index:**

blood test, 29

bradykinin, 16

brain injuries, 33

bone marrow transplantation, 23

Α C actin, 40 action potentials, 37 cancer, 2 activation, 35 activities of daily living, 50 capsule, 53 aged, 50, 51 carcinoma, 37 AIDS, 10, 46 cardiac surgical procedures, 40 alcohol, 46 cardiology, 25 alcohol virulogy, 45 cardiovascular disorders, 7, 30 alcoholic liver disease, 23 cardiovascular system, 31 alcoholism, 8, 10 carotid sinus, 7 Alzheimer's dementia, 33 CD4 cell, 20 androgens, 35 cell death, 2 angiotensin, 19 cerebrovascular circulation, 48 angiotensin converting enzyme, 16 cervical cancer, 48 anti-arrhythmia agents, 25 Chagas disease, 29 antibiotics, 52 chemotherapy, 37 antibody, 29 chlorides, 28 antibody response, 4 cholecystitis, 12 antimalarials, 36 cholesterol, 20 antimicrobial resistance, 17 chronic granulomatous disease, 40 apoptosis, 2, 8 chronic hepatitis, 43 arrhythmias, 37 cirrhosis, 23, 43 arteriosclerosis, 20 clinical outcomes, 41 articular cartilage, 6 clinical trials, 37, 46 asbestos, 27 cohort studies, 8 asthma, 38 colonic haustra, 44 atherosclerosis, 7, 33 compliance, 17, 21 autoimmunity, 4, 35 congestive heart failure, 19 coronary artery bypass, 40 coronary circulation, 13 В coronary disease, 41 coronary microcirculation, 13 B cells, 4, 30 coronin, 40 bacteria, 26 cytidylyltransferase, 36 bacterial DNA, 35, 36 cytochrome P-450, 13 bactericidal, 52 cytokines, 2, 20, 22, 30 baroreceptors, 47 baroreceptor reflex, 48 D biliary tract diseases, 12 biochemistry, 16, 33, 39, 56 biomechanics, 7 deacylases, 52, 53 biosynthesis, 39 dehydration, 50 blood coagulation, 33 depression, 21 blood pressure, 7, 48 diabetes, 3, 24, 39, 45, 56

breast cancer, 44

diagnosis, 29

diabetes mellitus, 13, 15

diabetic neuropathy, 56

diabetic vascular disease, 56

DNA, 2, 24, 35 gram-negative bacteria, 52 DNA, bacterial, 11 gram-positive bacteria, 52 granule proteins, 39 granulocytes, 39 granuloma, 38 growth, 33 growth factors, 16 guidelines, 17 Ε elastase, 5 Н elastin, 38 HCV, 45 electrophysiology, 25, 32, 37 emphysema, 38 health services research, 21 endocrinology, 4, 56 health status, 42 endothelium, 4, 13, 24, 33 heart myocardium, 37 endothelium, vascular, 31 Helicobacter pylori, 1 endothelium-derived relaxing factor, 13 hepatitis C, 23, 43, 45 endotoxin, 52, 53 hepatitis G, 43 endotoxin-binding proteins, 53 hip dislocation, 7 eosinophils, 38 hip prosthesis, 7 epithelial cells, 14 HIV, 10 esophageal motility disorders, 12 homocysteine, 33 esophagitis, 12 hospital mortality, 40 esophagus, 12 hospitals (veterans), 40 estrogens, 31 host defense, 39 extracellular matrix, 6 human immunodeficiency virus, 46 eye disease, 28 hydroxyl radical, 5, 6 hyperglycemia, 56 hypertension, 7, 30 F Fas. 8 ı fibromyalgia, 55 folic acid, 33 IgA, 14 fungal infection, 39 IGF binding proteins, 4 IGFs, 4 IL-1, 27 G immunity, 14, 23, 26, 36 immunodeficiency, 9 gallium, 6 immunohistochemistry solitary nucleus, 47 gastric injury, 18 immunology, 2, 4, 20, 23, 30, 42, 54 gastric motility, 18 immunotherapy, 2, 34 gastroenterology, 12, 18, 20 implementation, 17 gene expression, 24 infection, 21 gene regulation, 48 infectious diseases, 5, 31, 39, 42, 54 gene therapy, 25 inflammation, 1, 16, 22, 52, 53 genetic abnormalities, 15 insulin, 45 genetic engineering, 29 insulin resistance, human, 24 genetics, 55 interferon, 23, 43 glaucoma, 51 interferon-gamma, 14 glucose, 44, 45 interferon-gamma, recombinant, 11 glucose transport, 44 interleukin-12, 11 glutamic acid, 47 interleukin-8, 14

interleukin-r, 14 intestinal flow, 44 intestine, 20 iodides, 28 ion channel, 32 ion transport, 28, 46 iron, 5, 6 ischemia, 37

# K

kidney, 15 kidney calculi, 28 kidney tubule, 28

#### L

LAK cells, 2 Leishmania, 54 leishmaniasis, 54 leptin, 45 leukemia, 23 leukocytes, 31 L-fucose, 56 lipooligosaccharides, 53 lipoproteins, 53 liver disease, 23, 43, 45 long-term care, 49 lung, 5 lymphocyte, 2 lymphocyte activation, 30 lymphoma, 4 lysosomal proteins, 39 lysosomes, 39

# М

macrophages, 1, 6, 27, 31, 42 membranes, 52 metabolism, 4, 56 microbiology, 24 military environment, 42 molecular biology, 4, 16 monocytes, 31 mood disorder, 21 mortality, 8 motion perception, 51 mucosal, 14 multi-method research, 17 multimodality therapy, 37 muscle, smooth, 12, 44

muscle, smooth, vascular, 16 myeloma, 9 myeloperoxidase, 39

NaCl excretion, 46

# Ν

NADPH oxidase, 40 natural killer cells, 10 neisseria meningitidis, 53 neoplasms, 24 nephrology, 15, 28, 30, 46 nerve regeneration, 33 neurology, 33, 48 neurons, 33 neuro-ophthalmology, 28 neuropeptides, 19 neuropharmacology, 47 neurotransmitters, 47, 48 neutrophils, 1, 5, 31, 39, 40, 52 nitric oxide, 12, 24, 47, 48 NIV, 46 NK cells, 2 nosocomial pathogens, 17 nursing assessment, 50

# 0

obesity, 45 obstruction, 44 oncogenes, 24 ophthalmology, 28 organizational factors, 17 osteoarthritis, 6 outcome assessment, 8, 40 outcomes of care, 17 outer membrane, 53 oxalates, 28 oxidant stress, 24

# Р

papillomavirus, 24, 48, 54
parasitic diseases, 38
parasitology, 54
parasympathetic nervous system, 48
Parkinson's disease, 33
pathology, 24, 27, 48
patient satisfaction, 40
perimetry, 51
peroxidase, 38

Persian Gulf, 54 Persian Gulf War, 55 phagocyte, 6 phagocytosis, 1, 40, 42, 52 pharmacology, 32 phospholipase D, 31 phospholipases, 52 phospholipids, 52 photomedicine, 26 plasma, 23 pons, 48 potassium channels, 25 pressoreceptors, 7 programmed cell death, 8 prostaglandins, 30 prostate cancer, 18, 34 prostatic neoplasms, 8 protein malnutrition, 26 protozoa, 54 pseudomonas, 5 pseudomonas aeurginosa, 14 psychiatry, 21 PTSD, 42 pulmonology, 27 pupillary light reflex, 28 pyocyanin, 5 pyocyanine, 14

Q

quality of health care, 40

radiation therapy, 37

R

radical prostatectomy, 18 radioisotope therapy, 27 rape, 42 reactive oxygen species, 38 receptor, 45 receptors, 16 reflex, 47 renal function, 15 renal pelvis, 30 renal sympathetic nerves, 15 respiratory diseases, 27 retinoids, 38 rheumatoid arthritis, 27 rheumatology, 2, 4, 23

risk assessment, 40 risk factors, 40 RNA, 35 RNA sequence, 45

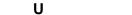
S

sensory receptors, 30 sepsis, 52 serum, 23 sex hormones, 31 siderophore, 5 signal transduction, 14, 16, 22, 31, 32 silica, 27 skin, 26 skin neoplasms, 26 solitary nucleus, 48 staphylococcus aureus, 52 steroid hormones, 46 stress, 18, 55 stroke, 25 substance P, 30 superoxide, 12 surfactant, 36 surgery, 12, 18 sympathetic nervous system, 7 symptomatic treatment, 18

Т

telemedicine, 49 therapy, 35 thrombin, 33 thrombomodulin, 33 thrombosis, 33 TNF, 27 TNF-alpha, 8 tolerance, 20 TRAIL, 8 transcription, 30 transcription, genetic, 54 transferrin, 5, 6 transmembrane signaling, 4 transplantation, 23 treatment, 21, 54 tropoelastin, 38 Trypanosoma cruzi, 29 tuberculosis, 42 tumor necrosis factor, 36

T lymphocyte, 9, 10



ulcers, 1 ureteral obstruction, 30 urine, 50 urocanic acid, 26

V

vaccine, 54
vaccines, 34
vascular dysfunction, 3
vasospasm, 25
veterans, 55
virology, 4
virus, 23
virus replication, 54

viruses, 54 vision, 28 visual field, 51 visual testing, 51 vocational impairment, 42

W

whole blood, 23 women, 42 wound healing, 21, 49 wound infection, 21

Υ

YY1, 21